ESSENTIALS
OF
PATHOLOGICAL CHEMISTRY

V. C. MYERS and M. S. FINE

NEW YORK
1913

ESSENTIALS
OF
PATHOLOGICAL CHEMISTRY

Including Description of the Chemical Methods Employed in Medical Diagnosis

BY

VICTOR C. MYERS, M.A., PhD., and MORRIS S. FINE, Ph.D.

PROFESSOR OF PATHOLOGICAL CHEMISTRY, NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL

INSTRUCTOR IN PATHOLOGICAL CHEMISTRY, NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL

Reprinted from
The Post-Graduate, 1912-13
New York
1913

PREFACE

The individual chapters of this volume are reprinted from the *Post-Graduate*, in which they have appeared at intervals during the past year. They have been designed primarily as a guide for the classes in Elementary Pathological Chemistry in this laboratory, although the tests described are likewise employed in the examination of the routine hospital specimens.

The various chapters have been written with the idea that an adequate appreciation of *normal physiological functions*, is a necessary prerequisite to an intelligent conception of *pathological chemical processes*, especially since the latter may be regarded as deviations from the normal. It will be observed that many of our diagnostic tests have for their purpose the detection of an inability on the part of the body to complete or properly carry out some of the normal physiological transformations. In harmony with this view, a consideration of the physiology of digestion in Chapters I and II is followed by the abnormal variations frequently encountered, and methods by which such abnormalities may be detected. Likewise Chapters V and VI deal with the normal mechanism of carbohydrate and fat metabolism, before indicating how certain functional defects may lead to glucosuria and acetonuria. As will be observed, the general arrangement of the chapters follows the course of the various food materials through the body.

Each chapter is composed of a resumé of the subject with which it deals, followed by directions for laboratory tests, especially those which are considered of diagnostic value. The authors have endeavored to make the discussions complete, though concise, presenting the subject with the most recent developments in mind. The " Laboratory Procedures " contain descriptions of tests, which for the most part are very simple and require only a limited equipment. However, tests and determinations are occasionally included which are not especially simple and necessitate considerable manipulation, but which, nevertheless, frequently yield valuable data. This is true of the methods for

blood analysis described in Chapter X. In the instances where several tests have been described, preference is indicated by the order of description.

In the preparation of this volume the authors have been guided primarily by the recent literature upon the subjects discussed, though frequent reference has been made to many of the standard texts on this and allied subjects, a list of which has been given on page 130.

The authors take this opportunity of acknowledging the kind cooperation of Dr. Jonathan Wright, Director of the Department of the Laboratories, not only in the preparation of this guide for their classes but also in the various other undertakings of the laboratory. They also desire to express their appreciation to the Editors of the *Post-Graduate*, Drs. C. G. Heyd and O. S. Hillman, for their kindness and advice in the publication of the different chapters, and to Dr. Ludwig Kast for valuable suggestions. The drawings and microphotographs of urinary sediments and photographs of apparatus were made for us by Mr. K. K. Bosse. For the reproduction of Fig. 14, we are indebted to the *New York Medical Journal*.

V. C. M.
M. S. F.

New York,
July, 1913.

CONTENTS

ESSENTIALS OF PATHOLOGICAL CHEMISTRY.

CHAPTER I.

Gastric Digestion Including Methods of Gastric Analysis.

Man ingests daily varying amounts of protein, fat and carbohydrate, together with water and inorganic salts. The quantities of these constituents depend upon the age, habits and occupation of the individual; sufficient food, primarily carbohydrate and fat, being necessary to supply the body with the required energy for heat and muscular work. This amounts to about 40 calories per kilogram of body weight, including sufficient protein to build up the tissues and to make good the wear and tear of the body. Just the quantity of this protein food essential for our daily needs is not quite clear at the present time. The dietaries of Voit and of Atwater call for upwards of 125 grams of protein, but Chittenden has recently pointed out that the body can be maintained in perfect health on half this amount.

Whatever this food intake may be, to render service to the body, it must undergo numerous changes, both before and after its absorption. The first step in the digestion of the food, aside from the mechanical factor of mastication, is the action of the ptyalin of the saliva upon the starchy foods with the production of dextrins and maltose, this action being long continued in the fundic portion of the stomach. With the taking of food into the mouth, normally a psychic secretion of gastric juice is started, and with the taking of certain substances into the stomach, *e.g.*, meat extracts, soup, etc., the secretion is augmented, and again still further increased by certain of the products of digestion. The essential constituents of this secretion are the hydrochloric acid (in about four-tenths per cent. concentration) and the enzymes, pepsin and rennin. The curdling action of rennin on milk is well known, though just its function in digestion is not so clear.

The pepsin, secreted in the inactive form, pepsinogen, and activated by the hydrochloric acid, produces important preliminary transformations in the protein. After the latter has been acted upon by the HCl and caused to swell, hydrolytic cleavage of the protein is begun, and normally before leaving the stomach, it has been broken down into proteoses and peptones. It will be well to bear in mind that gastric digestion together with pancreatic and intestinal digestion has much more to do than to render the food material soluble. Without exception, the protein, fat or carbohydrate is broken down into the simplest cleavage products, which are then resynthesized to form the body protein, body fat or glycogen.

Another factor of great importance is the mechanical one, upon which the X-ray observations of Cannon[1] have recently thrown much light. The stomach is always only so large as its contents, the function of the fundic portion being mainly that of a reservoir. A few minutes after the entrance of food into the stomach, peristaltic waves start in the prepyloric portion and run towards the pylorus. When a sufficient concentration of the hydrochloric acid has been reached, the pyloric sphincter relaxes and the advancing constrictions squeeze some of the material into the duodenum. The presence of the hydrochloric acid in the duodenum again causes the pyloric sphincter to close, and it is obvious that the waves running to the closed pylorus serve to thoroughly mix the food with the digestive juice. It is worthy of note that secretion of the gastric juice takes place in this region over which the peristaltic waves pass. Of the foods passing the pylorus, the protein should normally be found largely in the proteose-peptone stage, and the starchy material in the dextrin stage, but the fat will be found essentially unaltered. Practically no absorption takes place in the stomach under ordinary conditions.

During the course of 24 hours, a very large amount of gastric juice is secreted, probably over two liters. However, in the non-digesting stomach, only a very small amount of fluid, one to sixty cc. is found to be present under normal conditions, though pathologically large amounts are sometimes observed. The clinical examination of the gastric juice is generally made after the secre-

1. Cf. Cannon: The Mechanical Factors of Digestion, London and New York, 1911.

tion has been excited to activity by some definite test meal for a definite length of time. The test meal of Ewald-Boas, described below, is the one quite universally employed. The normal gastric contents as here obtained with the stomach tube is a white or light brown fluid with finely divided or pulpy bread in the sediment. The odor is not strongly sour, and the amount of mucus is scanty.

Volume.—The quantity of fluid obtained at the end of an hour after the Ewald test meal may normally vary between 50 and 100 cc. Larger amounts, 200 to 300 cc., are indicative of diminished motility or hypersecretion, while very large amounts, 500 to 4000 cc., suggest dilatation of the stomach, and usually benign or malignant stenosis of the pylorus.

Acidity.—The amount of free HCl found by the Töpfer method (see below) after a test meal in normal individuals averages between 30 and 40, but as high as 80 or 90 may be observed in certain pathological conditions, while in others, HCl may be entirely absent. The secretion of HCl is decreased in a variety of chronic diseases, including carcinoma of the stomach and advanced chronic gastritis; while in neurasthenic and hysterical individuals, it may be entirely absent. On the other hand, an hyperchlorhydria may be met with in a beginning gastritis, in continuous hypersecretion and in certain cases of gastric ulcer.

The total acidity usually lies between 50 and 80, and is dependent upon somewhat the same factors as the free HCl. The combined HCl averages 10 to 15, and together with the free HCl, represents the useful acid secretion.

The absence of the HCl affords rather favorable opportunity for bacterial action in the stomach, especially of a fermentative nature. Lactic acid is a very common product of this bacterial fermentation. Its presence in large amounts is suggestive, though not pathognomonic, of carcinoma. Quite generally, this large amount of lactic acid is accompanied by the presence of the Boas-Oppler bacillus, which probably plays an important part in the lactic fermentation. This organism, probably identical with the Bulgarian bacillus, is found in 75 to 85 per cent. of the cases of carcinoma of the stomach but seldom in other conditions. Volatile fatty acids, chiefly butyric and acetic, sometimes accompany fermentative con-

ditions of the stomach, but are of much less clinical significance than the lactic acid.

Enzymes.—The absence of the gastric enzymes, pepsin and rennin, is distinctly less common than that of HCl. In practically all cases in which sufficient HCl is present, there is an abundance of pepsin. The estimation of pepsin is chiefly of value in cases suggesting an advanced lesion of the gastric mucosa, and in which free HCl has been shown to be absent. The results of Rose[1] indicate that this is an important diagnostic test in carcinoma of the stomach.

Blood.—Hemorrhage from the stomach may be observed in the most diverse conditions. It may have a primary origin as in ulcer and carcinoma, or appear secondarily to diseases of other organs, leading to a hyperemic condition of the gastric mucosa.

Motility.—Considerable information, as stated above, may be obtained with regard to the motility of the stomach from the Ewald meal. If the activity is excessive, little or no gastric contents will be obtained; while if the motor activity is diminished or the organ is dilated, a large quantity of fluid will be obtained. To secure more accurate data in this regard, it is quite customary to resort to some retention meal, and in that way obtain a more accurate idea with regard to the retention of food in the stomach. In place of the regular evening meal, the patient may be given a plate of porridge, cooked with rice or raisins, and one or two slices of bread and butter, or as is employed in this hospital, four ounces each of boiled string beans and rice.[2] At seven or eight a.m. the next morning, the stomach is aspirated and the return examined for evidences of retention. Normally there should be no retention of food. The acidity will be found to correspond quite well with that of the Ewald meal, which is conveniently given subsequently to the removal of this retention meal.

Test-Meal.—The test-breakfast of Ewald and Boas is used almost exclusively in the examination of gastric juice. This consists of two slices of bread without butter, a glass of water,

1. Rose: *Arch. Int. Med.*, 1910, V., p. 459.

2. Many practical suggestions with regard to various laboratory tests have recently been given by Coffen: POST-GRADUATE, 1911, XXVI, p. 274.

and a cup of tea without milk or sugar; or, more accurately, about 50 grams of bread and 400 cc. of fluid. The test-breakfast should be eaten by the patient on an empty stomach, allowing ten minutes for its consumption, and exactly one hour later, should be siphoned from the stomach by an ordinary soft rubber tube.

LABORATORY PROCEDURES.

Before filtering the contents of the stomach, the general physical characteristics named below should be noted and recorded:

1. *Color.*—Recorded with the idea of possible detection of blood or bile.

2. *Consistency.*—Noted with special reference to possible presence of increased amounts of mucus.

3. *Odor.*—Whether normal (faintly sour) or fetid (rancid).

4. *Mucus.*—Detected by the consistency.

5. *Sediment.*

a. *Quantity.*—Whether the meal has been well or poorly digested. If well digested, there should be a layer of finely-divided bread residue on the bottom of the glass containing the stomach contents, and over this should be a layer of semi-transparent gastric juice; if poorly digested, the stomach contents will consist of only a small quantity of fluid and many coarse lumps of bread.

b. *Character.*—Whether blood, pus or stagnant remnants of food are mixed with the test-breakfast.

6. *Volume.*—The total volume is measured in cc. and then filtered through a folded filter preparatory to the chemical examination.

7. *Detection of Free Hydrochloric Acid.*—The presence of free HCl is very readily detected with a drop of congo red[1] or of Töpfer's[2] reagent, the congo red being turned blue and Töpfer's reagent a bright cherry red in the presence of this acid. For qualitative tests the reagents are conveniently used in the form of test papers.

8. *Quantitative Determination of the Acidity.*—If the *free acidity*, HCl, and *total acidity* are the only results desired, these

1. One-half gram of congo red dissolved in 90 cc. of water and 10 cc. of 95 per cent. alcohol added.

2. One-half gram of dimethylaminoazobenzene dissolved in 100 cc. of 95 per cent. alcohol.

figures can very easily be determined in the same solution. Ten cc. (or five cc. if necessary) of the filtered gastric contents are introduced into a porcelain dish and the *free acidity* titrated with N/10 NaOH[1], using three drops of Töpfer's reagent as indicator. When the initial pinkish-red color has been replaced by a bright yellow color, the reading is taken, and three drops of a one per cent. alcoholic solution of phenolphthalein[2] added. The solution is titrated until a distinct pink color reappears (total acidity).

Töpfer's Method.—Occasionally it may be desirable to determine the *combined acidity* and *acidity due to organic acids and acid salts*, in addition to the total and free acidity. Three 10 cc. portions are titrated with N/10 NaOH, using for indicators:

1. The physician will find it most convenient to secure the alkali of proper strength already prepared, but where considerable quantities are needed, sodium hydroxide of N/10 strength may be accurately prepared by a simple formaldehyde titration method from N/10 ammonium sulphate. Ammonium sulphate is an anhydrous salt and can be obtained perfectly pure.

a. 3.304 gms. of ammonium sulphate (one-fortieth of the molecular weight) are dissolved in distilled water and made up to exactly 500 cc. in a volumetric flask.

b. A few drops of a one per cent. alcoholic solution of phenolphthalein are added to 50-100 cc. of commercial formalin and dilute sodium hydroxide added until any formic acid present is neutralized, indicated by the appearance of the first permanent faint pink color.

c. About 4 grams of sodium hydroxide are dissolved in about 800 cc. distilled water, and a portion placed in a burette.

d. *Exactly* 10 cc. of the N/10 ammonium sulphate are now pipetted into a beaker, five cc. of the neutralized formalin added and the solution titrated with the sodium hydroxide to the first permanent pink. This process is repeated 5 to 10 times until one is sure of the exact strength of the hydroxide, and the required amount of water added to the remainder of the 800 cc. to dilute to decinormal strength.

When a neutral solution of an ammonium salt is treated with formaldehyde, combination occurs with the formation of hexamethylenetetramine (urotropin) and the liberation of a corresponding amount of titratable acid.

From the N/10 alkali, N/10 hydrochloric acid may be prepared.

A series of gravimetric determinations, which we have made upon N/10 sulphuric acid standardized to N/10 sodium hydroxide prepared in this way, has shown that this method is sufficiently accurate. For example, 25 cc. of N/10 sulphuric acid should give 0.2918 gm. of barium sulphate. Eleven determinations resulted as follows: 0.2928, 0.2918, 0.2922, 0.2912, 0.2924, 0.2920, 0.2928, 0.2920, 0.2914, 0.2926, 0.2918 gm. giving an average of 0.2921 gm., an error of one-tenth of one per cent.

2. One gram of phenolphthalein dissolved in 100 cc. of 95 per cent. alcohol

a. Töpfer's reagent (free acidity),
b. Alizarin[1] (combined acidity),
c. Phenolphthalein (total acidity).

In the case of the alizarin titration, the red color, which appears after the tinge of yellow (due to the addition of the indicator) has disappeared, must be replaced by a distinct violet color. Inasmuch as the alizarin reacts with all but the combined acidity, this acidity is obtained by subtracting the alizarin result from that of the phenolphthalein.

The various acidities are generally expressed by the number of cc. of N/10 NaOH necessary to neutralize 100 cc. of gastric juice.

9. *Lactic Acid.*—In the absence of free HCl, fermentation may take place in the stomach with the formation of lactic acid.

a. *Kelling Method.*—To a test-tubeful of water, a drop or two of 10 per cent. ferric chloride is added, so that the liquid is barely colored. One-half is then poured into a second tube and serves as a control. A small amount of the gastric filtrate is added to the other specimen, when in the presence of lactic acid, a distinct yellow develops at once, which appears the more marked when compared with the nearly colorless control.

b. *Strauss Method.*—To detect lactic acid, it is always preferable to remove it from disturbing factors, and the Strauss method also permits of a rough estimation of the amount of lactic acid present. Five cc. of gastric contents are placed in a small graduated separatory funnel, 20 cc. of ether added, and the mixture thoroughly shaken. As soon as the ether has separated, the solution is allowed to run out, except the uppermost five cc. of ether. Twenty cc. of water and two drops of 10 per cent. ferric chloride are added and the whole gently shaken. One-tenth per cent. of lactic acid gives a very intense yellowish-green color; five-hundredths per cent. a slight green color.

10. *Test for Pepsin.*—Pepsin may be detected quite simply as follows: The acidity of 10 cc. of filtered gastric contents is brought up to 0.2 to 0.5 per cent. with HCl and a flock of fibrin or bit of coagulated egg white added to this tube and a control of two-tenths per cent. HCl. The tubes are placed in the incubator

1. One gram of sodium alizarin sulphonate dissolved in 100 cc. of water.

for one to two hours and any digestion observed. The quantitative test below is nearly as simple and much more satisfactory.

11. *Estimation of Pepsin.*—The method of Rose[1] is both convenient and accurate. The solutions required are:

a. 0.25 gm. of pea globulin dissolved in 100 cc. of 10 per cent. sodium chloride solution.[2]

b. 0.6 per cent. hydrochloric acid solution.[3]

c. A measured volume of stomach contents neutralized to litmus paper with dilute alkali and diluted to five times the original volume with water; the gastric contents thus diluted is divided into two parts, one of which is boiled.

The determination is carried out as follows: In each of a series of six test tubes is placed one cc. of the globulin solution and one cc. of the acid. The unboiled gastric juice is then added in increasing amounts, with the final addition of the boiled juice to render equal the volume of fluid in each tube. The following scheme will illustrate this arrangement:

Tubes		1	2	3	4	5	6
Globulin solution.....	cc.	1.0	1.0	1.0	1.0	1.0	1.0
Hydrochloric acid.....	cc.	1.0	1.0	1.0	1.0	1.0	1.0
Unboiled gastric juice...	cc.	0	0.1	0.3	0.5	0.8	1.0
Boiled gastric juice.....	cc.	1.0	0.9	0.7	0.5	0.2	0
Total volume.....	cc.	3.0	3.0	3.0	3.0	3.0	3.0
Total acidity.....per cent HCl		0.2	0.2	0.2	0.2	0.2	0.2

The tubes are then shaken and incubated at 50 to 52° C. for 15 minutes or at 35 to 36° C. for one hour. *The enzyme content is expressed by the number of cc. of the globulin solution that would be digested by one cc. of the undiluted gastric juice under the above conditions.* Normally an enzyme content of 8 to 11 may be obtained.

1. Rose: *Arch. Int. Med.*, 1910, V, p. 459.

2. Pea globulin may be prepared by grinding up a couple of handfuls of garden peas, extracting with about 200 cc. of 10 per cent. sodium chloride filtering and then pouring the filtrate into about a liter of distilled water. The insoluble pea globulin settles to the bottom and is filtered off and dried at a low temperature. A 0.25 per cent. solution of this is now prepared in 10 per cent. sodium chloride.

3. Hydrochloric acid of 0.6 per cent. may easily be prepared from conc. hydrochloric acid (36.5 per cent.) by diluting two cc. of the conc. acid to 120 cc. with water.

In carcinoma values as low as three may be observed or in fact there may be no digestion at all.

12. *Rennin.*—Three to five drops of the gastric contents are added to 10 cc. of milk, and the mixture warmed to about 35° C., either in a water-bath or in an incubator. If coagulation takes place in fifteen minutes rennin is present in moderate amounts.

13. *Products of Digestion.*—Proteoses and peptones may be inferred in the presence of free HCl and pepsin, and the extent of starch digestion may be ascertained with dilute iodine and Benedict's solution.

14. *Blood.*—If the microscope has not revealed erythrocytes, the guaiac or other chemical tests may be applied preferably upon the retention when a retention meal has also been employed. To a small amount of the sediment, add an equal volume of 30 per cent. acetic acid and extract with ether. If the blood is present in considerable amount, the ether will assume a brownish-red color. Filter off the ether extract and to a portion of the filtrate, add 10 drops of an alcoholic solution of guaiac[1] and 20 to 30 drops of old turpentine or hydrogen peroxide. In the presence of blood, a blue color is produced. Other reagents, as benzidine, phenolphthalin or aloin may be employed,[2] but this test is very satisfactory and sufficiently delicate.

15. *Diagnostic Tests for Carcinoma of the Stomach.*—Various tests have been proposed as specific for carcinoma of the stomach, but none of these tests can be said to have been found entirely trustworthy. Futhermore, these tests do not react positively until the disease is quite well developed clinically. Two tests which are perhaps worthy of mention are the Salomon test and the Neubauer-Fischer tryptophane test.

a. *Salomon Test*[3].—The principle underlying this test is the fact that carcinomata secrete protein, which becomes mixed with the gastric contents. The diet of the patient for 24 hours prior to the test should be free from soluble protein. At the beginning of this period he is given a morning meal of milk and gruel and a mid-day meal of boullion with coffee or tea. Late in the evening, the stomach should be washed out with large quantities of pure water until

1. One gram of good guaiac resin dissolved in 60 cc. of 95 per cent. alcohol.

2. See Chapter II, p. 24.

3. Salomon: *Deutsch. med. Wochschr.*, 1903, XXIX, p. 547.

the return water is clear. The following morning the fasting stomach is washed twice with 400 cc. of physiological salt solution, the same solution being used each time. The total nitrogen and protein are estimated in this wash water by the Kjeldahl and Esbach methods respectively. (For methods, see chapters on urine). Salomon found in cases of gastric carcinoma, 20 to 70 mgms. of nitrogen and from 0.00625 to 0.05 gms. protein to each 100 cc. of fluid. In non-malignant cases, no protein was found, and the nitrogen varied from 0 to 16 mgms.

b. *Tryptophane Test.*—This test was first suggested by Neubauer and Fischer[1] with the use of glycyltryptophane. Weinstein[2] entirely dispensed with the expensive glycyltryptophane, and simply tested the juice obtained after a regular meal. This technique has been found to be less reliable than the original test, though Jacque and Woodyatt[3] have recently observed that a solution of Witte's peptone is nearly as efficient as glycyltryptophane. The modified procedure is as follows: Four to five hours after a regular meal some stomach contents are secured and filtered. About 5 cc. of the juice are then mixed with an equal volume of sterile 2 per cent. Witte's peptone, toluene added, the tube shaken, and incubated at body temperature for 24 hours. At the end of this time three to four cc. of the mixture are taken and if not acid, treated with a few drops of three per cent. acetic acid. Bromine water[4] is now added drop by drop, until in case the reaction is positive, a reddish violet color appears.

This test is based on the fact that carcinomatous tissue contains an enzyme of stronger proteolytic power than pepsin, causing the appearance of amino acids, including tryptophane. Blood and trypsin must not be present. (The latter may be regarded as absent if bile has not been detected).

Smithies[5] in the Mayo Clinic has found that more than one-third of the proved cases of cancer of the stomach gave the glycyltryptophane test, more than one fourth were lactic acid

1. Neubauer and Fischer: *Deutsch. Arch. f. klin. Med.*, 1909, XCVII, p. 499.
2. Weinstein: *Jour. Amer. Med. Assoc.*, 1910, LV, p. 1085.
3. Jacque and Woodyatt: *Arch. Int. Med.*, 1912, X, p. 560.
4. Water to which sufficient bromine has been added to saturate it.
5. Smithies: *Arch. Int. Med.*, 1912, X, p. 357.

positive, while about one-thirteenth gave the Weinstein tryptophane test.

16. *Microscopical Examination.*—This is to be made upon the sediment of the retention meal or better still upon fluid removed from the fasting stomach, looking particularly for leucocytes, erythrocytes, starch grains, bacilli (especially the Boas-Oppler bacillus) and tumor particles.

CHAPTER II.

DIGESTIVE CHANGES IN THE INTESTINE, TOGETHER WITH THE FORMATION, COMPOSITION AND CLINICAL SIGNIFICANCE OF THE FECES.

It will be recalled that food materials which have passed the pyloric sphincter into the duodenum, are normally composed of the proteins, quite largely in the proteose-peptone stage of hydrolysis, the starchy material, in the dextrin stage, while the fatty foods have encountered practically no change. The hydrochloric acid of the chyme, when present in the duodenum, acts upon a substance termed prosecretin, transforming it into secretin, and the latter, through the medium of the blood, stimulates a discharge of bile and a secretion of pancreatic juice. The pancreatic juice is an alkaline fluid and contains amylolytic, lipolytic, and proteolytic enzymes, *i.e.*, enzymes which act upon all three types of food stuffs. The amylopsin of the pancreas has a stronger action upon starch digestion than the ptyalin, and converts any unattacked starch and the dextrins to maltose. Likewise the trypsinogen, after its activation by the enterokinase of the intestinal juice, breaks down any unaltered protein into proteoses and peptones, and to a considerable extent, splits these up into their constituent amino acids. The action of the pancreatic lipase is likewise one of hydrolytic cleavage, in which the fats are split into their simplest components, the fatty acids and glycerol, in which transformation the lipase is greatly facilitated by the bile salts. Digestion has now by no means been completed. In the secretion of the intestinal juices, enzymes are to be found which carry out many of the final transformations. Before they are ready for absorption, the disaccharides, maltose, lactose and sucrose, must be broken down to the monosaccharides. In the *succus entericus* are found the enzymes, maltase, lactase and sucrase, for this purpose. The proteoses and peptones which have escaped complete hydrolysis by the trypsin will find the enzyme, erepsin, waiting to complete this action. Thus we observe that digestion really consists in

the breaking down of our various food materials into the smallest possible integral parts or "Bausteine." From these, our body carbohydrate, fat and protein are constructed. The monosaccharides, of which glucose is the chief, are picked up by the portal circulation and carried to the liver, where the sugar, over and above approximately one tenth of one per cent, is stored up as glycogen. The recent researches of Folin make it evident that the amino acids themselves are picked up by the blood, but just where their synthesis into body protein, or the deamidization of the acids not employed in tissue repair takes place is not clear. Fat appears to be reformed from the fatty acids and glycerol by the epithelial cells of the intestinal wall which absorb these cleavage products. It is then picked up by the lacteals, and, in the form of a very fine emulsion (chyle), carried by the lymphatics to the thoracic duct, and there emptied into the blood stream.

The food materials are propelled along the intestine by the same peristaltic movements that have previously been noted in the esophagus and stomach. A series of advancing waves of constriction, preceded by waves of relaxation, serve to propel the masses of food material. In addition to the peristaltic wave, a second type of movement is observed in the small intestines, viz., a series of local constrictions occurring rhythmically at those points at which masses of food lie. The apparent purpose of this is to thoroughly mix the material with the digestive secretions and bring it in intimate contact with the absorptive walls. The consistency of the fluid passing the ileocecal valve is about the same as that leaving the stomach. The greater part of the nutritive materials are absorbed while they are being passed along the small intestines. The absorption of the excess of water takes place in the colon, however, and is aided by waves of antiperistalsis passing at intervals over the ascending portion. In the descending colon peristaltic waves are again observed which carry the feces toward the rectum.

The reaction of food material as it passes through the body undergoes many changes. The strongly acid character of the chyme, as it leaves the pyloric sphincter, is soon changed to a faint alkalinity by the alkaline pancreatic juice. However, when the reaction of the fluid passing the ileocecal valve is taken, it will be found to be slightly acid, due to bacterial fermentation, but again bacterial changes in the colon render the material

discharged from the rectum neutral or even faintly alkaline to litmus.

The fact that about one-third of the dry matter of normal human feces consists of bacteria, and at least one-half of the nitrogen of the feces is bacterial in its origin[1] serves to emphasize the importance of bacteria in the intestinal canal, though experimental evidence would indicate that the presence of this large number of bacteria is a normal and even useful condition. In nurslings, the bacterial flora is relatively simple, though later in life, the number of these bacterial forms becomes very large. The dominant organism in nurslings is *B. bifidus*, but this is ultimately replaced by *B. coli* and *B. lactis aerogenes*. Other organisms which may be observed are *coccal forms*, *B. aerogenes capsulatus*, and, in certain cases, *B. putrificus*. These last two organisms Herter[2] is inclined to associate with conditions of excessive putrefaction in the intestines. In early life, the products of intestinal decomposition are remarkably small in amount, and, as would be expected, the number of putrefactive bacteria are few. One finds, however, in middle life a large number of persons in whom the putrefactive conditions in the intestine are distinctly more active than was the case earlier in life. Unquestionably the most important factors in bringing about this strongly proteolyzing type of bacterial flora are the consumption of an overabundance of protein food, combined with inadequacy in the digestive juices, delayed absorption, and insufficient motility in the alimentary canal. Very little decomposition takes place in the large intestine under the action of *B. coli*, if the absorption in the small intestine has been good.

It is easy to comprehend that urinary constituents, such as urea, uric acid, creatinine, etc. are derived from the metabolism of protein in the body, whether the protein be the body's own, or that of an animal fed to it, but the intestinal canal, where the feces are formed, is a long tube open at both ends, through which may pass the nitrogen gas of the air swallowed and various indigestible materials. In diarrhea, the curds of milk, pieces of undigested meat or bread, and large quantities of fat are in evidence. These com-

1. Schmidt and Strasburger: Die Fäzes des Menschen, 3rd. edit., Berlin, 1910, p. 327; MacNeal, Latzer and Kerr: *Jour. Inf. Dis.*, 1909, VI., p. 123; Matill and Hawk: *Arch. Int. Med.*, 1911, XIV., p. 433.

2. Herter: The Common Bacterial Infections of the Digestive Tract, 1907; Cf. also MacNeal, Latzer and Kerr: *Jour. Inf. Dis.*, 1909, VI., p. 571.

mon observations would seem to justify the general supposition that normal feces are made up of undigested food residues. On the contrary, this is far from the fact. The feces are chiefly the unabsorbed residues of intestinal secretions. Furthermore, as Mendel and others have shown, the feces is the normal path for the elimination of a number of the important inorganic elements, such as iron, calcium, etc. As a proof that feces are a true secretion, it has been shown by F. Voit that the material secreted in an isolated loop of the intestine of a dog is of a similar composition, and contains the same amount of nitrogen as the feces of the normal intestine through which food is passing. Prausnitz defines normal feces as those resulting from the eating of any food that is completely digested and absorbed. It is entirely probable that on a diet whose constituents are not entirely available, the amount of feces is increased by the undigested cellulose, and the nitrogen content is increased by the large amount of digestive juices secreted, because of the large volume of food and the accompanying increased peristalsis. As pointed out above, a large part of the dry matter of human feces consists of bacterial substance. A larger part of the organic material eliminated in the feces is, however, of unknown nature and composition.

Amount.—Upon an ordinary mixed diet, the daily fecal excretion of an adult male will average 110 to 170 grams, with a solid content varying between 25 and 45 grams. The feces of such an individual upon a vegetable diet will be much greater and may even amount to 350 grams and possess a solid content of 75 grams. The variation in the normal daily output being so great renders this factor of little value for diagnostic purposes, except where the composition of the diet is accurately known. Lesions of the digestive tract, a defective absorptive function, or increased peristalsis, as well as admixture of mucus, pus, blood and pathological products of the intestinal wall may cause the total amount of excrement to be markedly increased.

Consistency.—The form and consistency of the stool is dependent, in large measure, upon the nature of the diet. Under normal conditions the consistency may vary from a thin, pasty discharge to a firmly formed stool. Stools which are exceedingly thin and watery ordinarily have a pathological significance.

Color.—The fecal pigment of the normal adult is hydrobilirubin, also called stercobilin. It originates from the bilirubin

which is secreted into the intestine in the bile, being formed by the reducing activity of certain bacteria. Hydrobilirubin bears a close resemblance to urobilin and may even be identical with that pigment. Neither bilirubin nor biliverdin occurs normally in the fecal discharge of adults, although the former may be detected in the excrement of nursing infants. The diet is the most important factor in determining the color of the fecal discharge. A mixed diet, for instance, produces stools which vary in color from light to dark brown; an exclusive meat diet gives rise to a brownish black stool, whereas the stool resulting from a milk diet is invariably light colored. That certain drugs act to color the fecal discharge is well illustrated by the occurrence of green stools following the use of calomel, and of black stools after bismuth ingestion.

Odor.—The odor of normal feces is generally stated to be due to skatole and indole. However, these aromatic putrefactive substances are generally found in such small amounts as to form an insufficient explanation on this point. Hydrogen sulphide no doubt plays a certain part in the disagreeable character of the odor. The intensity of the odor depends to a large degree upon the kind of diet, being very marked in stools from a meat diet, much less marked in stools from a vegetable diet, and frequently hardly detectable in stools from a milk diet. Thus the stool of the infant is ordinarily odorless, and any decided odor may generally be traced to some pathological source.

Reaction.—Normal stools generally have a neutral reaction, although slightly alkaline or even acid stools are met with. The acid reaction is encountered much less frequently than the alkaline and then commonly only following a vegetable diet.

A simple division of fecal material may be based upon the separation afforded by the customary procedures, viz., the estimation of the total nitrogen, ethereal extract, and carbohydrate residues. The results obtained with these methods have yielded data of great scientific importance, though the time required and the nature of the results obtained often render them of rather small value diagnostically.

Nitrogenous Substances.—Three sources are usually considered as contributing to the nitrogenous material excreted in the feces; food residues, residues of the digestive juices and cellular material from the intestinal wall, and bacteria and their products. The

quantity of this nitrogen amounts to from one-half to two grams and from four to eight per cent. of the dry feces. Upon a meat diet the food residues represent almost nothing under normal conditions, *i.e.*, the muscle protein is practically 100 per cent. utilized[1]. In the case of vegetable proteins it has been a matter of common observation that the utilization was not as good as with animal proteins. This in part at least is explained by the inaccessability of certain of the vegetable proteins to the digestive juices, for as Mendel and Fine[2] have shown, the proteins of the wheat, and probably also of the barley and corn are as well utilized as meat, when taken in a pure form or freed from extraneous cellular substance. With legumes the utilization was not quite as good, though this point they are as yet unable to fully explain. That about one-half the fecal nitrogen is derived from the fecal bacteria has already been pointed out. A great variety of nitrogenous substances may be formed by bacterial action upon the protein or its cleavage products. Among such may be mentioned indole, skatole, phenol, indole acetic acid, various oxy-acids, in certain instances putrescine and cadaverine, etc. That intoxication may result from poisonous products formed by bacterial action can hardly be questioned, though just what the substances are that exert this effect cannot be stated at the present time.[3] Much attention has been devoted to the products of bacterial action on tryptophane, viz., indole acetic acid (urorosein), skatole and indole. The presence of a large amount of indican in the urine is no doubt indicative of increased intestinal putrefaction. It is questionable, however, whether indole in the amounts absorbed in this way has any toxic properties.

With regard to the elimination of fecal nitrogen under pathological conditions, observations[4] show that it is increased in biliary obstruction, intestinal fermentative dyspepsia, and diarrhea; and decreased in chronic constipation.

Ethereal Extract.—The bodies which go to make up this ethereal extract are the neutral fats, free fatty acids, (and fatty acids

1. Cf. Mendel and Fine: *Jour. Biol. Chem.*, 1912, XI, p. 22.
2. Mendel and Fine: *Jour. Biol. Chem.*, 1911, X, pp. 303, 399, 345, 433.
3. Myers, Fisher and Diefendorf: *Amer. Jour. Insanity*, 1909, LXV, p. 607; also *Zentr. f. Stoffwechsels*, 1908, IX., p. 849.
4. Schmidt und Strasburger; Die Fäzes des Menschen, p. 130.

in the form of soaps when an acidified solvent has been employed), and koprosterol formed from cholesterol by the action of reducing bacteria. This ethereal extract ordinarily forms from 12 to 25 per cent. of the dry weight of the feces. The utilization of fat varies under normal conditions from 90 to 95 per cent. depending upon the source of food. The higher fats such as stearin are much less readily assimilated. In biliary obstruction as much as 70 grams of fat may be eliminated in the feces, forming 50 per cent. of the dry weight of the material. In various conditions associated with defective fat digestion (pancreatic disease) or defective fat absorption increased amounts may be eliminated, while in chronic constipation the amount may be decreased. In both biliary obstruction and pancreatic disease the fat utilization has been found to be as low as 25 per cent.

Carbohydrate Residues.—Normally feces may yield on hydrolysis reducing substance equivalent to from one-half to two grams of glucose or from two to six per cent. of the dry weight of the feces. The utilization of the carbohydrate is regarded to be about 98 per cent. Ordinarily starch digestion does not seem to be interfered with, though the amount of carbohydrate material eliminated in the severer catarrhal conditions of the intestine may be slightly increased. One question to be asked with regard to all carbohydrate material is, are the enzymes of the alimentary canal capable of hydrolyzing it. There appear to be no enzymes in the digestive tract capable of attacking certain of the more complex carbohydrates[1].

To determine the functional capacity of the intestine, a test-diet may be administered on the same general principles as this is employed in connection with gastric activity. The following test-diet has been suggested by Schmidt and Strasburger[2].

In the morning, one-half liter of milk (or tea, or cocoa, if possible with much milk) together with one roll and butter, and one soft-boiled egg; *for breakfast*, one dish of oatmeal-gruel, cooked in milk and strained (salt or sugar permissible); *at noon*, one-quarter pound finely chopped lean beef, broiled rare, with butter,

1. Mendel: *Zentr. f. Stoffwechsels*, 1908, IX, p. 641.

2. Schmidt and Strasburger: Die Fäzes des Menschen, 1910, also Schmidt: The Examinations of the Function of the Intestines by Means of the Test Diet, 2nd Amer. Edit. by Aaron, Phila., 1909, p. 11.

the interior raw, along with it, not too small a portion of potato broth (well strained); *in the afternoon*, the same as in the morning but no egg; *in the evening*, one-half liter of milk, or one plate of soup (as in the morning), together with a roll and butter, and one or two soft boiled eggs.

The diet in a healthy individual should yield the normal feces, according to the idea of Prausnitz, *i.e.*, it should have practically no food residues and be almost entirely "metabolic" in origin. This diet may be varied slightly, depending upon the information desired. The essential ingredients are the milk, white bread, potato broth and half-raw meat to show various possible deficiencies. The diet employed in this hospital consists of the following: *for breakfast*, tea or coffee with cream, farina and two eggs; *for lunch*, chopped meat cooked as above, mashed potatoes and white bread; *for dinner*, rice or macaroni, toast or zwieback with butter, milk and one or two eggs.

For quantitative work, a detailed test-diet, such as that originally suggested by Schmidt and Strasburger[1] which contains about 110 grams protein, 105 grams fat and 200 grams carbohydrate, should be employed. For making the functional test, the patient is kept on the diet for three days, or even longer, until a stool is obtained which is certainly derived from it. Under normal conditions, this occurs at the second defecation after the beginning of the test. To mark this point, the patient must take a five grain capsule of willow charcoal. This substance passes through the intestine unaltered and marks off the corresponding point in the feces. In this way, also both the beginning and the end of a period may be determined, so that if feces are desired for the whole of a period, for example, three days, they may easily be separated.

Many of the valuable hints obtained from the examination of the feces following the test diet are mentioned in connection with the laboratory tests. Some of the more salient features will bear discussion here. The appearance of mucus in the feces indicates the existence of an inflammatory condition of the mucous membrane. Fatty stools may have their origin in a biliary obstruction or deficiency, a disturbance of pancreatic secretion, or a disturbance of intestinal digestion.[2] If connective tissue-

1. Cf. Schmidt-Aaron: *Loc. cit.*, p. 13.
2. Schmidt-Aaron: *Loc. cit.*, p. 39.

remains appear in the feces, it is a sign of disturbance in gastric digestion. The gastric juice alone can digest raw connective tissue. If macroscopic muscle-remains appear in the feces, this is a sign of a disturbance of digestion in the small intestines. Many valuable hints with regard to carbohydrate and also protein remains are obtained from the incubator test.

The so-called nuclei test of Schmidt is perhaps as reliable a test for pancreatic inefficiency as any we possess. One-half cm. cubes of beef or thymus[1] are hardened in alcohol, but previous to use washed for several hours in water, then tied in a silk gauze bag, and served with the test diet. If pancreatic secretion is suspended, nuclei will be found in the silk gauze bag which appears in the stool, and can easily be recognized with the aid of the microscope.

Laboratory Procedures

Feces may be very conveniently collected for examination in glass, screw top jars about four inches high and four inches in diameter. If the purpose of the examination is for the functional examination of the alimentary tract, the feces should be obtained after a diet such as that of Schmidt and Strasburger mentioned above. All specimens should be examined as soon as possible after evacuation.

1. *Macroscopic Examination.*—This forms the most important part of the whole procedure, and alone is often sufficient to enable the experienced observer to form a judgment. In the first place, it determines whether color, consistency and odor correspond with the normal feces.

a. *Color.*—Milk produces light brown feces; cocoa, reddish brown; absence of bile, or any condition producing a large amount of fat, gives clay-colored stools; blood from the upper part of the alimentary tract yields "tar feces." Fermentation feces are light brown and foamy, while putrefactive feces are dark.

b. *Odor.*—Mildly excremental (normal); butyric acid-like (fermentation); malodorous (putrefactive).

1. The use of thymus cubes was suggested by Einhorn and because of the great abundance of nuclei is to be preferred to the beef. Further with Einhorn's bead test, the test diet may be entirely dispensed with. Einhorn discusses the important facts connected with his test in a recent number of the Post-Graduate, May 1912, XXVII., p. 359.

c. *Consistency.*—The feces may be hard, well-formed, with wide bore; thin and soft; soft with admixture of hard lumps; diarrheal; they may contain mucus, intimately mixed with feces, or easily separated from the same; undigested matter, *e.g.*, fruit stones, vegetable skins, intestinal gravel, gall-stones, parasites, etc.

Under pathological conditions, such food-remains as the following may appear: remains of connective tissue and tendons from the chopped meat eaten, remains of muscle tissue, potato remains (glassy, transparent granules, which appear like sago-grain), and fat remains. Other consitutents which may be observed are: mucus, in large and small flakes, and large crystals of ammonium-magnesium phosphate.

2. *Microchemical Examination.*—This serves for the most part to supplement the macroscopic examination. The feces are thoroughly mixed, and a portion as large as a walnut transferred to a mortar and ground with water to a thin mush. A drop is transferred to each of four slides, or the four drops may be placed upon the four parts of the same slide. Both the low ($\frac{2}{3}$) and high ($\frac{1}{6}$) power objectives are employed in this examination.

a. The *first drop* is simply covered with a cover glass. It is examined for muscle fibers, noting their relative abundance and state of preservation, connective tissue remains, yellow fatty acid salts of calcium, colorless soaps, drops of neutral fat, mucus particles, leucocytes, erythrocytes, vegetable cells, etc.

b. The *second drop* is throughly mixed with a drop of 36 per cent. acetic acid and heated until bubbles appear. It is covered with a cover glass and examined for fatty acid flakes. This treatment also serves to differentiate between connective tissue remnants and mucus, the former being rendered transparent.

c. The *third drop* is treated with a couple of drops of a saturated 70 per cent. alcoholic solution of Sudan III, which stains the fat globules red.

d. The *fourth drop* is rubbed up with a drop of iodine solution.[1] It is examined for starch granules, fungi, yeast cells, etc. (colored blue or violet).

1. The iodine solution is prepared by adding sufficient iodine to a 2 per cent. potassium iodide solution to color it a light brown.

The following conditions are pathologic: In " a," fragments of muscle in large numbers and good state of preservation, needles of fatty acid and soap, drops of neutral fat, numerous groups of potato cells, parasite eggs, mucus, connective tissue, pus, etc.; in " b," massive fatty acid flakes; in " c," many red fat globules, and in " d," many blue colored remains of starch granules, blue or violet fungous spores and yellow yeast cells.

3. *Reaction.*—The reaction is taken with strips of red and blue litmus paper upon feces ground up with distilled water.

4. *Schmidt's Sublimate Test.*—Some feces rubbed up with water, are placed in saturated mercuric chloride, stirred, and allowed to stand over night. Normal feces are colored red (hydrobilirubin.) A green color (even if confined to microscopical particles) is pathological (unchanged bilirubin). In occlusion of the bile duct, no color is obtained.

5. *Fermentation Test.*—From the fresh excrement, which has been stirred up, but not yet thinned with water, a small amount, about the size of a walnut, is taken with a wooden spatula, and put into the lower vessel *a* of Strasburger's fermentation tube.[1] (In hard stools less is taken; in soft, more; in fluid stools, the lower vessel is entirely filled). In this, it is stirred up with water with the aid of a wooden spatula, and then the rubber stopper is put on, care being taken to exclude air bubbles. The rubber stopper is now taken from the little tube *b* and the tube filled with tap-water. This is then closed by turning over the vessels *a* together with the small stoppers, and the vessel *c* (empty) connected with it; *c* has an opening on top and acts as an ascension pipe. When the apparatus is put together, it is placed for 24 hours in an incubator heated to 37° C. If gas is developed from the feces within this time, this collects in *a* or *b*, and a corresponding amount of water is driven into the ascension pipe *c*. The height of the water in the ascension pipe is noted, the vessel *a* is opened, and the reaction of it is tested with litmus paper, and compared with the reaction before the test was instituted.

Normally, very little or no gas is found, and the original reaction of the feces does not change materially. If *c* is filled one-half or more, pathological conditions are indicated. If at the same time, the reaction has become decidedly more acid, carbohydrate fermentation has taken place (the positive result of

1. See Fig. 13, Appendix.

the fermentation test), which is further indicated by the light color of the feces. If the reaction has become more alkaline and the color is dark, putrefaction is indicated. Both fermentation and putrefaction may occur at the same time, in which case the reaction may be neutral.

6. *Dissolved Albumin.*—From 20 to 30 grams of the feces are ground up with water, which should be added gradually in small quantities, until thinned into a fluid consistency. It is allowed to stand for several hours and then filtered through a double filter. The muddy filtrate is clarified with some pure diatomaceous earth. To this clear filtrate, 36 per cent. acetic acid is added, drop by drop, as long as a turbidity or a precipitate is formed. This precipitate is the (*normal*) nucleoprotein of the feces. The material is again filtered with the aid of diatomaceous earth, and the new filtrate is tested with a drop of potassium ferrocyanide solution to ascertain the presence of dissolved albumin (and albumose).

The examination for dissolved albumin comes into consideration only in cases of diarrhea, and then only when it is questionable whether the fundamental disturbance is purely functional or organic. It is a matter of observation that a diarrheal stool, which contains no mucus, but shows a reaction for albumin and putrefies in the incubator test, is mixed with transudate serum, and, therefore, points to an irritated or inflammatory condition, or to ulcers of the mucous membrane.

7. "*Occult*" *Blood.*—The test for blood is of value only in cases of organic disease, and then it has for its condition a diet *without meat.* A number of tests for occult blood have been suggested, among such being the guaiac, benzidine and phenolphthalin tests, the last two being extremely delicate when properly applied. The guaiac test is, however, sufficiently delicate for most purposes.

a. *Guaiac Test.*—About 5 to 10 grams of feces are stirred in a mortar with water until it is a thick, fluid-like consistency. If the feces contain much fat, this should be extracted with about three volumes of ether and poured off. To this thick, fluid-like excrement is added one-third the volume of glacial acetic acid and thoroughly mixed. About 10 cc. of the entire mass are poured into a test tube, about the same amount of ether added, and the material carefully,

but thoroughly, mixed. The ether is then allowed to separate. This may be quickly accomplished with the aid ot the centrifuge. In the presence of blood, the spectroscope will reveal absorption bands of acid hematin. More simply, blood may be detected in this ethereal extract, which has been poured into another tube, by adding 10 drops of a one to sixty alcoholic solution of guaiac resin, followed by 20 to 30 drops of ozonized turpentine, or about four cc. of 10 per cent. hydrogen peroxide. The whole is shaken. A blue coloring shows the presence of blood, provided that there is no pus mixed with the feces.

b. *Benzidine Test.*—A knife point full of pure benzidine (must be kept in a dark place) is dissolved in two to three cc. of glacial acetic acid. To this, are added two cc. of hydrogen peroxide. A small amount of feces is now shaken up with a little hot water, and about three cc. of the suspension added to the above solution. If blood is present, a greenish or bluish reaction will occur in from one to three minutes. Delicacy 1–100,000.

c. *Phenolphthalin Test.*—On adding about one cc. of the phenolphthalin reagent[1] to a solution of a small bit of the suspected fecal matter in water (about two cc.) and treating with one, or, at most, two drops of a 10 per cent. solution of hydrogen peroxide, a bright red color will develop, owing to a reoxidation of phenolphthalin to phenolphthalein through the agency of the oxidase of the blood in the presence of the peroxide. Delicacy 1–800,000.

8. *Indentification of Gall-stones.*—Gall-stones are occasionally found in the feces after passage from the gall-bladder, but only after careful washing and sifting of the excreted material. Gall-stones are classified according to the amount of the various ingredients which they contain. In man, the biliary concretions are generally cholesterol calculi, or pigment calculi, or combinations of the two. The presence of these constituents serves to identify the calculus as biliary in origin.

1. The reagent is prepared as follows: To 100 cc. of a 20 per cent. solution of sodium hydroxide are added two grams of phenolphthalein and 10 grams of zinc dust. The bright red solution is heated gradually until it has become decolorized, or rather until it has assumed a slight yellowish tone, owing to a reduction of the phenolphthalein to phenolphthalin. The supernatant fluid is poured off into a colored glass bottle, and the access of air prevented by the addition of a little liquid paraffin which floats on top.

The calculus is pulverized in a clean dry mortar and extracted with ether. Upon filtering off the ether and allowing it to evaporate, any cholesterol will crystallize out. If some of the crystals are dissolved in chloroform and stratified upon concentrated sulphuric acid, the acid will take on a greenish fluorescence, and the chloroform will become red. The residue of the calculus, remaining after the ether extraction, is washed with dilute hydrochloric acid, dried, then extracted first with chloroform, and afterwards with hot alcohol. The chloroform will extract the bilirubin, and the alcohol the biliverdin, the former imparting a golden yellow color to the solution, the latter an emerald green.

9. *Examination of Duodenal Juice.*—Since the introduction of the Einhorn duodenal pump[1] making it possible to easily obtain fluid from the duodenum, interest has arisen in this fluid's being made of diagnostic value. As yet the examination of the duodenal juice[2] has not yielded data of decided value in diagnosis, though it would appear to be the most logical method of ascertaining a deficiency in the pancreatic secretion. Duodenal juice obtained in this way is normally a clear, golden yellow, slightly viscid fluid. It is neutral or faintly alkaline in reaction to litmus and has a specific gravity of about 1.005. The juice contains bile, as its color would indicate, and active amylolytic, lipolytic and proteolytic enzymes. The methods which we have employed for estimating the enzyme content are as follows:

a. *Amylase* (*amylopsin*).—The Wohlgemuth method is simple and fairly satisfactory. Into each of six small test tubes are introduced 5 cc. of 1 per cent. soluble starch solution. Tube 1 serves as a control and to the remaining five tubes are added .05, .1, .25, .5 and 1.0 cc. of the juice diluted one half with distilled water. The tubes are then incubated at 38° C for 30 minutes, *immediately* filled nearly full with cold water, several drops of N/10 iodine added and the tubes shaken. The tube is selected as positive which shows an entire disappearance of all blue color. The enzyme activity is expressed in the number of cc. of starch solution, 1 cc. of undiluted juice is capable of digesting. If it takes 1 cc. of juice to digest 5 cc. of starch, the activity is 5, if

1. Cf. Einhorn: *Diseases of the Stomach*, New York, 1911, p. 86.

2. Cf. Einhorn and Rosenbloom: *Arch Int. Med.*, 1910, VI, p. 666; Hess; *Amer. Jour. Dis. Child.*, 1912, IV. p. 205 and other papers; Crohn: *Amer, Jour. Med. Sci.* CXLV, 1913, p. 393.

digestion is accomplished by .25 cc., it is 20, etc. For the five tubes as diluted above the activity figures are 200, 100, 40, 20 and 10. The activity of different specimens has been found to vary between 5 and 200, although the average figure is about 40.

b. *Lipase* (*steapsin*).—Into each of two test tubes are introduced 1 cc. portions of the juice, one of which is boiled to serve as control. To each of these tubes are added 1 cc. of neutral ethylbutyrate, 10 cc. of distilled water and 1 cc. of toluene. The tubes are shaken and placed in the incubator at 38° C for 24 hours, shaking several times during the interval. At the *end* of this time, they are removed to porcelain dishes and titrated with N/20 NaOH, using phenolphthalein as indicator. The titration result of the boiled tube is subtracted from the unboiled to obtain the figure for the lipolytic action. We have obtained figures varying from .3 – 4.3 cc. The average has been 1.5 – 2.0.

c. *Protease* (*trypsin*).—The methods available for estimating trypsin are not as satisfactory as those for pepsin. Two methods will be described, the Gross casein method, and a modification of the Fermi gelatin method. Casein has the disadvantage that it is also attacked by erepsin, while the gelatin digestions must be carried on at room temperature.

Casein Method.—Into each of six small test tubes, as in the amylase method, are introduced 5 cc. of .1 per cent. pure casein in .1 per cent. sodium carbonate[1] and the same amounts of duodenal juices added as in the case of the amylase. The tubes are incubated for 15 minutes at 38° C and then acidified with a few drops of dilute acetic acid. The tube which remains perfectly clear, *i.e.*, in which digestion has been complete, is recorded. The tryptic activity may be calculated in the same way as the amylolytic activity above. These figures are exactly ten times those obtained according to the original Gross calculations, *i.e.*, the five tubes according to Gross represent an activity of 20, 10, 4, 2 and 1. By this method we have found the activity ordinarily to be 4–10.

Gelatin Method.—Into a small test tube is pipetted 1 cc. of undiluted duodenal juice and 3 cc. of water, 1 cc. of .5 per cent.

1. The soluble starch and casein solutions may be preserved with chloroform and toluene for some little time, especially if kept in a refrigerator.

sodium carbonate and 1 cc. of toluene added. Into the test tube is then inserted two 3 cm. gelatin tubes[1] and the tube allowed to incubate for 48 *hours at room temperature* with occasional *gentle* mixing. At the end of this time the amount of digestion is measured with a millimeter scale and the measurements added together. The average total digestion with this method in some fifty analyses has been 3.0—4.0 cm.

1. The gelatin tubes we have employed have been prepared by dissolving 10 grams of gelatin, 1 gram of sodium fluoride in distilled water, deeply coloring with a clear solution of cochineal and making up to 100 cc. and filling tubes of 2 mm. inside diameter. The tubes 3 cm. in length are cut just previous to use.

CHAPTER III.

The Physical Properties, Inorganic and Organic Physiological Constituents of Urine.

Since the end products of the metabolism of nitrogenous and mineral substances find their principal exit through the kidneys, a study of the secretion of these glands under various conditions may be expected to throw light upon the processes involved in the metabolism of the above substances. With a knowledge of the principal constituents of the urine and a partial understanding, at least, of their history in the body, the appearance of any unusual substance or the presence of a normally occurring constituent in an amount inconsistent with the attending conditions may not infrequently serve to detect derangements of body functions.

The mechanism of kidney secretion has been a much controverted question. Perhaps the most generally held view at present is that the renal epithelial cells actively participate in the secretion, the water and inorganic salts being eliminated in the capsular region, while the urea, uric acid, etc. find their exit through the uriniferous tubules.

Volume.—The volume of urine eliminated depends in great part upon the volume of fluid ingested. Under normal conditions one liter may be taken as the average volume of urine excreted in twenty-four hours. This, however, is subject to great variations under both normal and pathological conditions.

The secretory activity of the kidney is to a considerable extent controlled by its blood supply, the latter, in turn, being dependent upon general blood pressure and upon the state of constriction or dilatation of the renal vessels. Thus where the blood pressure is raised, as for example in chronic nephritis, and the blood supply to the kidneys is consequently augmented, one observes an increased secretion of urine. Digitalis may exert its influence in this manner, although it probably also produces a stimulating action upon the secreting cells of the kidney. The tendency of arterial pressure to increase the blood flow, and consequently to augment the urinary secretion, may be masked

by a constriction of the renal vessels. Such a condition obtains in strychnine or adrenaline poisoning, in asphyxia, eclampsia, etc. The flow of blood is increased when the renal vessels are dilated, although this may be counteracted by a general fall in pressure, in which case there would be no actual improvement in the renal circulation. Dilatation of the vessels of the kidney is the probable explanation for the large volume of urine eliminated in diabetes insipidus. A diminished blood flow through the kidneys may result from increased venous pressure—frequently associated with cardiac diseases.

The condition of the renal epithelial cells influences the volume of urine, the latter being usually diminished by lesions of these cells. However, in certain cases of nephritis the blood flow is increased, thus tending to augment the secretion of urine. It is thus apparent that in nephritis the volume of the secretion will be diminished or increased, depending upon which of these factors exerts the greater influence. Generally in acute nephritis the renal changes are sufficiently prominent to produce decreased secretion, while in chronic nephritis this tendency may be counteracted by the increased arterial pressure, although even here when cardiac failure ensues, as would be expected, the volume of urine eliminated is diminished.

The volume of urine is diminished by conditions which cause an increased elimination of water through other channels, for example through the alimentary tract during diarrhea or vomiting, or through the skin as perspiration. On the other hand during cold weather, when cutaneous evaporation is reduced, the volume of urine is increased. Thus in warm weather the volume may be as low as 400 cc., while a volume of 2000 may be encountered during cold weather.

When the kidneys are unable to properly excrete *e.g.*, salt, giving rise to edema, water is retained in the tissues to maintain normal osmotic relations. On the contrary, when it is necessary to eliminate a large amount of material as is the case with sugar in diabetes mellitus, the volume of urine is increased.

Opposition to the flow of urine may be encountered at any place along the urinary tract. Such obstruction may be due to scar tissue, calculi, tumors, etc. If but one kidney or ureter is thus affected, the total urine elimination may not be markedly influenced as the other kidney will very likely be able to perform the extra work thus thrown upon it.

Color.—The color of urine may vary under normal conditions from a very pale yellow to a reddish yellow, depending upon its density. Pathologically the color may vary from a light yellow to dark brown or black. A red color may be due to blood; very dark colored urines may arise after taking carbolic acid; the excretion of melanin from pigmented tumors may likewise be the cause of a dark color, especially after being exposed to the air for some time or on the addition of an oxidizing agent. A green or brownish yellow color may be due to bile. Ingestion of rhubarb, senna or santonin yields urine of yellow color, which becomes red on the addition of an alkali. In alkaptonuria—an anomaly of metabolism—the urine may become dark owing to the presence of homogentisic acid. This is especially so if the urine is allowed to become alkaline. Further attention is devoted to this topic in Chapter VII.

Specific Gravity.—The specific gravity of normal urine most commonly falls between 1.015 and 1.025. It may, however, be as low as 1.010 or as high as 1.040 without necessarily indicating pathological conditions. In general, both normally and pathologically, the specific gravity is inversely proportional to the volume. In diabetes mellitus, however, we may observe both a large volume and a high specific gravity owing to the presence of sugar.

Reaction.—In the majority of cases the urine is acid to litmus. This is due to the usual preponderance of "acid radicals" over "basic radicals." The acid radicals take their origin in the metabolism of proteins, etc., during which are produced the sulphuric acid and a part of the phosphoric acid (from the oxidation of the sulphur and phosphorus of the protein), and organic acids, such as hippuric, uric and oxalic acids. The "basic radicals"—sodium, potassium, calcium, magnesium and ammonia—of the body are called upon to partially neutralize these acids. Ordinarily the latter are not completely neutralized, thus accounting for the customary acid nature of the urine. The reaction of the urine may experience marked changes under both physiological and pathological conditions.

An animal dietary yields a preponderance of acid-forming substances, while on a vegetable diet the base forming elements are usually in excess.[1] Thus an highly acid urine is usually

1. Sherman and Gettler: *Jour. Biol. Chem.*, 1912, XI, p. 323.

associated with animal food; and a diet containing much vegetable material may yield neutral or even alkaline urine. The foregoing most likely accounts for the fact that the urine of dogs is normally acid, while that of rabbits is habitually alkaline. That this difference may be attributed to the diet is shown by the fact that a dog, subsisting on a vegetable dietary may excrete an alkaline urine, while a rabbit, metabolizing animal material (*e.g.*, in starvation) may eliminate an acid urine.

The development of excessive acidity, owing to the ingestion of difficultly oxidizable acids (mineral acids), or the pathological formation of acids (as in diabetes), is counteracted in a measure by the neutralizing action of the bases, sodium, potassium, calcium and magnesium. When the acidity is so great that an adequate supply of these elements can no longer be economically furnished by the body, ammonia is called upon to meet this need. This accounts for the relative increase of ammonia in severe diabetes. The proximity to a meal may affect the reaction of the urine. For example, the secretion of hydrochloric acid into the stomach during the process of digestion may so reduce the store of acids in the body, that for a time after a meal, the urine may be neutral or even alkaline, giving rise to the so-called " alkaline tide."

Unless certain precautions are taken, the urine sooner or later after voiding, becomes alkaline owing to the conversion of urea into ammonium carbonate by bacteria. In cystitis this decomposition may take place in the bladder. If a urine is alkaline immediately after voiding, one should decide whether the alkalinity is due to fixed alkali or to ammonia. Only in the latter event is cystitis indicated. An alkaline urine may be the result of absorption and excretion of alkaline transudates.

From the foregoing, it is apparent that the reaction of the urine is determined by the character of the diet, proximity to a meal and presence or absence of ammonia-producing organisms in the urine either before or after voiding.

Odor.—Normal urine has a characteristic aromatic odor. The excretion of certain drugs (cubebs, copaiba, myrtol, saffron, tolu and turpentine) imparts specific odors to the urine. When the latter has undergone alkaline fermentation, a disagreeable ammoniacal odor is developed.

Transparency.—When voided the urine of a normal individual

is usually perfectly clear. On standing a few hours, a cloud or "nubecula" forms, even in normal urine. This cloud consists of mucus threads, epithelial cells, etc., from the urinary passages. Under pathological conditions, the latter may be greatly increased and accompanied by casts or blood. If the acidity of the urine is somewhat diminished (as after a meal) a turbidity due to phosphates may form. This will disappear on adding a little acetic acid. On standing in the cold, urates may settle out but will again go into solution on warming.

Chlorides.—Under ordinary conditions 10 to 15 grams of sodium chloride are excreted daily. These figures are, however, greatly dependent upon the salt intake. In starvation the sodium chloride excretion is reduced to a minimum. The same conditions obtain in cases of carcinoma of the stomach, resulting in stenosis of the pylorus, essentially a condition of starvation. The sodium chloride elimination is decreased by those conditions which favor its removal from the blood through other channels, *e.g.*, cases of diarrhea, rapidly formed transudates and exudates, such as pleurisy with effusion. It may be pointed out that for several days after the reabsorption of an exudate, the chloride excretion may be greatly increased, and is here a favorable diagnostic sign. Diminished chloride elimination is observed during the crises of acute febrile diseases, especially pneumonia and in chronic nephritis, in the latter case probably because of the relative impermeability of the kidney to salts. In febrile diseases it is worthy of note that the elimination of chlorides progressively decreases as the febrile process approaches its crisis, and tends to rise to its original level during convalescence. The chloride elimination appears to be augmented by exercise, by copious water drinking, and in diabetes insipidus.

Phosphates.—The average excretion of P_2O_5 is one to five grams daily. This originates to a small extent from the oxidation of the phosphorus of protein material. It owes its origin in greater part to the phosphate of the food, and the extent to which the latter controls the phosphate excretion in the urine depends upon the relative abundance of alkali and alkali-earth phosphates. The alkali-earth phosphates are difficultly absorbable and hence are in great part eliminated directly through the feces, thus contributing but little to urinary phosphates. The alkali phosphates are absorbed and add to urinary

phosphates to a greater extent, but even these may be converted into alkali-earth phosphates in the body and be in part excreted into the intestine, reappearing in the feces. The phosphate elimination is said to be increased in periostosis, osteomalacia, rickets and after copious water drinking; and decreased in acute infectious diseases, pregnancy and diseases of the kidney. At times a turbidity due to phosphates is observed. This is frequently erroneously interpreted as indicating an increased elimination of phosphates, " phosphaturia." It is more likely due to a condition of decreased acidity and is more properly termed " alkalinuria." This precipitation of phosphates may also be due to an unusual amount of calcium which would form one of the less soluble phosphate combinations.

Sulphates.—Sulphur is excreted in three forms: *oxidized* or *inorganic sulphur*, *e.g.*, the sulphates of sodium, potassium, calcium and magnesium; *ethereal sulphur*, *e.g.*, sulphates of phenol, indoxyl, skatoxyl, cresol, etc.; *neutral sulphur*, *e.g.*, cystine, cysteine, taurine, hydrogen sulphide, etc. The greater part of the sulphur of the urine is present in the oxidized or inorganic form, averaging about 2.5 grams calculated as sulphuric acid daily, this as a rule being about ten times the amount of ethereal sulphur excreted. The inorganic sulphur of the urine arises mainly from the oxidation of the sulphur of protein material, and is thus increased by those conditions which stimulate protein metabolism as acute febrile diseases and decreased when the rate of metabolism is lowered. The ethereal sulphates of the urine are increased by excessive formation and absorption from the intestine of products of putrefaction, *e.g.*, phenol, indole, skatole, or by the administration of similar aromatic bodies such as phenol, cresol, resorcinol.

Sodium, Potassium, Calcium and Magnesium.—The quantities of these elements appearing in the urine are subject to great variations under normal conditions, and are greatly dependent upon their concentrations in the food. Very little is known regarding their pathological variations. Giving approximate figures it may be said that the urine contains daily five grams sodium (as Na_2O); three grams potassium (as K_2O); and about one gram of calcium and magnesium phosphate together. Calcium salts are in great part excreted through the intestine, which condition makes the calcium concentration of the urine an unreliable index to the extent of absorption of calcium compounds.

Ammonia.—Under ordinary conditions the nitrogen of ammonia, in combination with urinary acids, is present in the urine to the extent of 2.5 to 4.5 per cent. of the total nitrogen eliminated, *i.e.*, about 0.7 grams per day. A considerable portion of this represents ammonia which has escaped conversion into urea so that it might be utilized to neutralize the sulphuric, phosphoric, uric acids, etc., formed in the process of normal metabolism or introduced with the food. This procedure probably operates to prevent undue drain upon the body's supply of sodium, potassium, calcium and magnesium. If sufficient fixed alkalies or alkali-earths are administered, so that ammonia is not required for neutralizing the acids, then the ammonia excretion may be greatly reduced, or in fact as Janney has recently shown[1] almost completely disappear from the urine. Furthermore, as Sherman and Gettler[2] have demonstrated, the ammonia output is dependent to a considerable extent upon the balance between the acid-forming and base-forming elements of the foods. An injury to the liver cells results in an increased output of ammonia owing to the fact that these cells normally convert ammonium salts to urea. Increased elimination of ammonia has been observed in pernicious vomiting of pregnancy. It is important to note that here the individual is essentially in a condition of inanition, which itself is characterized by a relative increase in ammonia elimination.[3]

A very large number of organic compounds have been found in normal urine. For our present purposes the nitrogenous substances are of chief interest and of the latter urea, uric acid creatinine, creatine, hippuric acid and the purine bases will be considered. For a general survey of the state of protein metabolism, the determination of the total nitrogen suffices, and indeed is to be preferred to the estimation of urea. Although the latter represents the major portion of the total nitrogen (60 to 90 per cent.), nevertheless a knowledge of the amount of urea alone leaves one in ignorance as to the excretion of the 40-10 per cent. of nitrogen in other combinations. When more detailed or specific information concerning the condition of nitrogenous metabolism is desired, the various constituents above mentioned may be determined with profit.

1. Janney: *Zeitschr. f. physiol. Chem.*, 1912, LXXVI, p. 99.
2. Sherman and Gettler; *Jour. Biol. Chem.*, 1912, XI, p. 323.
3. Underhill and Rand: *Arch. Int. Med.*, 1910, V., p. 61.

Urea.—Urea is the chief end product of protein metabolism and its quantitative excretion is closely proportional to the amount of protein ingested. Thus variations of 10 to 40 grams are not uncommon. The percentage of urea is dependent upon the volume of urine in addition to the protein of the diet, and when it is considered that the former may vary from 500 cc. to 2000 cc., it is evident that but little information can be gained from a knowledge of merely the percentage of urea. The urea nitrogen in proportion to the total nitrogen excreted may likewise be greatly influenced by the amount of protein in the diet. Thus with a high protein intake, the urea nitrogen may make up as much as 90 per cent. of the total nitrogen; while with a diet containing relatively little protein but considerable carbohydrate and fat, the proportion may be as low as 60 per cent. With a nitrogen intake of 20 grams the urine would contain approximately 20 grams of nitrogen of which 18 grams may be in the form of urea; whereas with a nitrogen intake of seven grams the excretion of urea nitrogen may be as low as four grams.

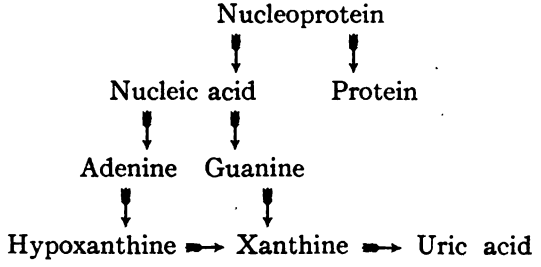
The origin of urea may be considered at this point. It is generally held that the amino acids formed during digestion are carried by the portal circulation to the liver and there those not needed for tissue repair (the greater part) are deamidized, *i.e.*, ammonia is split off. The latter uniting with the carbonic acid of the blood forms ammonium carbonate, which, in turn, is converted into ammonium carbamate and this finally into urea. Precisely the manner in which the amino acids are converted into urea is still a matter of dispute. Indeed Folin and Denis have recently shown that the amino acids are not deamidized in the intestinal wall or immediately in the liver, but may be carried unchanged directly to all parts of the body.[1] Nevertheless, it may be assumed that ammonia is ultimately split off and is normally converted into urea principally in the liver. Consequently any condition which hampers the liver cells from transforming ammonia into urea would be expected to lead to a diminished output of the latter and an increased elimination of the former. Thus in cirrhosis and in acute yellow atrophy, where the function of the hepatic cells is disturbed, the urea excretion may be abnormally low. On the other hand it must be noted that even in extensive alteration in the liver, the relation

1. Folin and Denis: *Jour. Biol. Chem.*, 1912, XI, p. 87; *ibid.* p. 161.

between urea and ammonia is frequently not essentially changed. A diminished urea elimination may likewise be found in such conditions as acidosis where considerable ammonia is utilized to neutralize acid substances before it can be transformed into urea. Impaired kidneys may result in a lowered output of urea although this is not a constant association. It may also be noted that in renal disease the curve for the urea elimination discloses eccentric and unaccountable variations. When the rate of metabolism is accelerated as in fevers, exopthalmic goitre, etc., the total nitrogen and urea are augmented.

As already pointed out, it is quite essential in considering the excretion of total nitrogen and urea to compare these values with the nitrogen of the food, because, only when the nitrogen output is out of proportion to the intake can an abnormal condition be presumed to exist.

Uric Acid.—Uric acid results from the cleavage and oxidation of nuclear material. Nucleoprotein is split into protein and nucleic acid. When the nucleoprotein is present in the food, this process takes place in the alimentary tract under the influence of trypsin; when the body cells are the source of the nucleoprotein this transformation takes place in the tissues probably through the agency of a similar enzyme. In either case, the nucleic acid yields the purine bases, adenine and guanine, through the agency of the enzyme nuclease. Adenine and guanine are then converted respectively into hypoxanthine and xanthine, this change being accomplished by the enzymes adenase and guanase. Finally by means of an oxidizing enzyme, xanthine is transformed into uric acid. This process may be represented as follows:[1]



1. For a more detailed consideration of these transformations see Myers; *Albany Med. Ann.*, 1911, XXXII, p. 645.

It has been claimed that in man about half the uric acid is further subjected to an enzymatic change (uricolysis), being partially converted to urea. This, however, is still a disputed question, although it undoubtedly takes place in dogs. A small amount of the above mentioned adenine, guanine, etc. escapes conversion into uric acid, and thus gives rise to the purine bases of the urine.

The precursors of uric acid—nucleoprotein and purine bases—may be present in the food or in the disintegrating cellular matter of the body. In the former case the uric acid is said to be of "exogenous origin;" in the latter of "endogenous origin." For practical purposes it may be stated that "endogenous" uric acid varies among individuals from 0.2 gram to 0.5 gram being fairly constant for a given individual and essentially independent of the the protein intake. Any uric acid in excess of this is to be attributed to substances of the food, which in the process of digestion and assimilation yield uric acid. Such food materials are meat, meat extracts, pancreas, liver, thymus, etc., also vegetable seed materials, *e.g.*, peas and beans. On a mixed diet 0.7 gram of uric acid may be taken as an average.

Ordinarily uric acid is present in the urine as sodium, potassium or ammonium urate. Only when the urine is especially acid does uric acid itself separate out. When the urine is concentrated or after the ingestion of considerable meat, pancreas, etc., urates may be deposited shortly after the urine is voided. In other cases such deposits may form on standing in a cool place. These urate sediments dissolve on warming.

The greatest increase in uric acid elimination is observed in leukemia, as much as 12 grams having been found to be excreted in the 24 hours. This high elimination of uric acid is without doubt to be referred to the enormous increase in the number of leucocytes and consequent leukolysis. An increased uric acid excretion is observed in other diseases associated with a high grade of leucocytosis.

Our knowledge of the relation of uric acid to gout is at present in an exceedingly unsettled condition and the question cannot be fully discussed here. We may say briefly that the quantitative excretion of uric acid in gouty individuals does not differ markedly from that found normally. It may, however, be noted that for

two or three days preceding an attack of acute gout the uric acid elimination is diminished; while during and for a few days after the attack it may maintain a level somewhat above normal.

The lithia and piperazine therapy has been employed with the object of presenting media in which uric acid and urates are more soluble than they are in blood or urine. However, it is unlikely that these substances will react with the uric acid any more readily than with the other acids of the body; and hence the portion of these therapeutic agents actually combining with the uric acid is probably insignificant. "Atophan" does indeed frequently increase the elimination of uric acid and is said to be helpful in acute gout. Nevertheless, whether or not this drug is of lasting benefit is an open question. It has been pointed out, for example, that atophan produces only a temporary increase of uric acid output, which is compensated by a subsequently lowered elimination.[1] This suggests, according to Dohrn, that atophan produces its initial effect by stimulating the formation of uric acid from its immediate precursors, and, when the store of the latter is exhausted, no further increase in the elimination of uric acid takes place—in fact a lowered output may result.

Creatinine.—Our accurate knowledge with regard to the elimination of creatinine is of very recent date, namely, since the introduction of the Folin colorimetric method for its estimation in 1904. During this interval, creatinine has perhaps received greater attention at the hands of investigators than any of the other nitrogenous urinary constituents.[2] Creatinine is very probably derived from the creatine of muscle or some common precursor substance, but just where or how this transformation takes place has not been ascertained. The quantity of creatinine eliminated is independent of either the amount of protein in the food or of the total nitrogen in the urine and is almost absolutely constant from hour to hour and from day to day for a given normal individual. This statement refers strictly speaking to an individual upon a creatinine and creatine free diet, though ordinarily the urinary creatinine is almost entirely endogenous in origin. Ingested creatinine quite largely re-

1. Cf. Dohrn: *Zeitschr. f. klin. Med.*, 1912, LXXIV, p. 445.

2. For reveiw of literature see Myers: *Amer. Jour. Med. Sci.*, 1910 CXXXIX, p. 256.

appears in the urine, but ingested creatine influences the creatinine elimination only slightly, if at all, and does not to any extent pass into the urine unchanged. This latter fact, first observed by Folin, has thrown a degree of doubt on the connection of these two bodies in metabolism. Neither decreased nor increased muscular activity uncomplicated by other factors has any effect upon the creatinine elimination. While the daily creatinine excretion is practically constant for each healthy individual, different persons excrete different amounts and Folin first noted that the chief factor determining this was the weight of the person. He further observed that the fatter the subject the less creatinine was excreted per kilo of body weight, and concluded from this that the amount of the creatinine excretion depended primarily upon the mass of active protoplasmic tissue. Benedict and Myers[1] found that the creatinine excreted by women was, in general much lower than in the case of men, doubtless due to their poorer muscular development. Creatinine is by far the most reliable index as to the amount of a certain kind of true tissue katabolism occurring daily in any given individual, and further appears to be an index of some special process of normal metabolism taking place largely if not entirely in the muscles. The intensity of this process appears to be associated with the muscular strength of the individual. Normally one to two grams of creatinine are eliminated daily. It has been found convenient to express this in milligrams of creatinine nitrogen per kilo of body weight, and this "creatinine coefficient" varies between 7 and 11 for a strictly normal individual.

A low creatinine elimination has been found to be associated with a large number of pathological conditions, especially those accompanied by muscular weakness, such as exophthalmic goitre, muscular dystrophy, carcinoma of the liver, etc. Benedict and Myers observed a creatinine coefficient as low as 2 in two very old, decrepit women. To a certain extent, the creatinine elimination appears to serve as an index to the physical condition of fitness of a given individual. In diseases where the creatinine output is lowered, creatine is generally excreted.

An increased creatinine elimination is observed in fevers, the rise in the excretion paralleling the rise in body temperature very closely, likewise the elimination of total nitrogen. The in-

1. Benedict and Myers: *Amer. Jour. Physiol.*, 1907, XVIII, p. 377.

creased excretion is entirely due to the hyperthermia, and creatinine is thus an index of the amount of the increased metabolism due solely to this cause, as pointed out by Myers and Volovic.[1] They have found in experimental fevers in rabbits, that the elimination is ordinarily increased about 35 per cent. Creatinine is still an index of the amount of a certain kind of endogenous metabolism, which is proceeding here at an abnormal intensity.

Creatine.—Creatine is a constant constituent of both striated and non-striated muscle, though it exists in much smaller quantities in the former. The creatine concentration of striated muscle appears to be both constant and distinctive for a given species.[2] Creatine does not appear to be a normal constituent of adult urine, though the recent results of Rose[3] have shown its presence in the urine during infancy and childhood. Benedict[4] was the first worker to note the appearance of creatine in the urine in considerable quantity, and that during inanition, an observation which has been abundantly confirmed. Subsequently Benedict and Myers[5] reported the elimination of creatine in a considerable number of pathological conditions, in which its appearance was to be accounted for by the poor nutritive condition of the patients. The elimination of creatine has since been noted in a variety of diseases, among which are exophthalmic goitre, in convalescence after typhoid fever, muscular dystrophy, anterior poliomyelitis, pernicious vomiting of pregnancy, carcinoma of the liver, etc. In the last mentioned disease the amount eliminated is very large, 1–1.5 grams. In many of these cases, notably fevers and hepatic carcinoma, under-nutrition is an important factor in the production of creatine. The appearance of creatine is generally found to be associated with a loss of muscle protein, thus indicating its source to be the creatine of muscle tissue. Recent experiments,[6] in which the decrease in the creatine store of the body during starvation has been accounted for by the amount of its excretion in the urine, appear to demonstrate this origin. When the muscle protein is used to supply abnormal demands made upon it (energy), the creatine of the urine is possibly an index of the

1. Myers and Volovic: *Jour. Biol. Chem.*, 1913, XIV, p. 489.
2. Myers and Fine: *ibid.*, 1913, XIV, p. 9.
3. Rose: ibid., 1911, X, p. 265.
4. Benedict: Carnegie Inst. Wash., 1907, Pub. No. 77, p. 386.
5. Benedict and Myers: *Amer. Jour. Physiol.*, 1907, XVIII, p. 407.
6. Myers and Fine: *Proc. Soc. Exp. Biol. Med.*, 1912, X, p. 12.

amount of this protein destruction. Substances which exert a sparing effect on protein metabolism, such as carbohydrates, might be expected to decrease or prevent the elimination of creatine. That carbohydrate alone will cause the creatine of the urine in starvation to disappear has been demonstrated.

Hippuric Acid.—On a mixed diet an average of 0.7 gram of hippuric acid is eliminated in the urine. Hippuric acid is a combination of glycocoll and benzoic acid. The former arises from the protein substances of the body; while the latter owes its origin in part to certain aromatic bodies of the food (vegetable) which are ultimately converted into benzoic acid, and in part to putrefactive changes in the intestine. Ingestion of benzoic acid itself or its salts increase the output of hippuric acid.

Oxalic Acid.—Oxalic acid in the form of calcium oxalate usually occurs in the urine in very small amounts, about 0.02 gram in 24 hours. Oxalic acid is probably formed from the metabolism of proteins and fat. Its output may be increased by ingesting foods which contain oxalic acid. Such foods are cabbage, spinach, apples, grapes, etc.

LABORATORY PROCEDURES.

When the examination is to be a quantitative one, it is imperative to collect a twenty-four hour sample, to which a preservative is added, such as toluene, thymol or chloroform. The color and specific gravity taken together with the volume are valuable as means of orientation, and may at times serve to indicate some unusual condition. Thus a small volume of highly colored urine usually has a relatively high specific gravity; while a low specific gravity is commonly associated with a large volume of pale urine. Occasionally a light colored urine with a high specific gravity is obtained—the characteristic type observed in diabetes. The reaction is noted by means of red and blue litmus paper. If alkaline, the nature of the alkalinity should be determined—whether due to fixed or volatile alkali. The latter condition may be accompanied by an ammoniacal odor. When the urine is not transparent, the turbidity or sediment is usually due to (a) urates, (b) phosphates, (c) bacteria, pus, epithelial cells, etc. If urates, the sediment will disappear on warming; phosphates dissolve on the addition of dilute acetic acid; while the turbidity due to the formed elements will not be affected under this treatment.

1. *Volume.*—This is conveniently measured in a one or two liter graduated cylinder.

2. *Color.*—The color is recorded following some such color scheme as that of the Vogel scale in which the colors are pale yellow, light yellow, yellow, reddish yellow(amber), yellowish red (deep amber), red, brownish red, reddish brown, brownish black, black.

3. *Specific Gravity.*—This is ordinarily ascertained with the aid of the urinometer. A large test tube, or the cylinder usually accompanying the urinometer is three-fourths filled with urine. The urinometer is then allowed to sink in the fluid and the reading taken from the lower portion of the meniscus in contact with the stem of the instrument. If the observation is not made at the temperature for which the instrument is calibrated, a correction should be made when an accurate observation is necessary as in the calculation of total solids or in the estimation of sugar by the specific gravity method. Most instruments are graduated to be read at 15°C. The correction for temperature is roughly made, by adding 0.001 for every 3°C above this 15°C and the same subtracted for every 3°C below.

4. *Total Solids.*—The quantity of the total solids eliminated in the twenty-four hours may be approximately computed by multiplying the second and third decimal figures of the specific gravity by 2.6. This gives the number of grams of solids in one liter of urine. From this the 24 hour amount may be calculated.

5. *Chlorides.*—(Volhard-Harvey Method)[1]. Five cubic centimeters of urine are pipetted into a small porcelain evaporating dish and diluted with about 20 cc. of distilled water. The chlorides are now precipitated with exactly 10 cc. of the standard silver nitrate solution[2] and about two cc. of the indicator[3] added. Standard ammonium sulphocyanate solution[4] is then run in from

1. Harvey: *Arch. Int. Med.* 1910, VI, p. 12.

2. 29.06 grams of silver nitrate dissolved in and made up to one liter with distilled water. Each cubic centimeter of such a solution is equivalent to 0.01 gram of sodium chloride.

3. 100 grams of crystalline ferric ammonium sulphate dissolved in 100 cc. of 25 per cent. nitric acid.

4. Dissolve about 13 grams of ammonium sulphocyanate in 800 cc. of distilled water. According to the method described in the text, titrate this solution against the standard silver nitrate and estimate how much water should be added to the remainder of the sulphocyanate solution to make it exactly equivalent to the standard silver solution.

a burette until the first trace of yellow shows throughout the mixture. By subtracting the number of cubic centimeters of sulphocyanate thus employed from ten and multiplying by 0.01, the number of grams of sodium chloride in five cc. of urine are obtained. From this the total chloride output for the twenty-four hours may be computed.

6. *Phosphates.*—To 50 cc. of urine in a beaker or an evaporating dish are added five cc. of accessory solution[1] and the mixture heated to the boiling point. By means of a burette, a standard solution of uranium nitrate[2] is then run into the hot solution, until a drop of the mixture yields a brownish coloration when brought in contact with a 10 per cent. solution of potassium ferrocyanide on a porcelain plate. The number of grams of P_2O_5 in 50 cc. of urine is estimated by multiplying by 0.005 the amount of uranium nitrate solution required, and from this the quantity P_2O_5 in the total volume of urine can be readily calculated.

7. *Total Acidity and Ammonia.*—Both these determinations may be performed on the same sample—the former by the method of Folin and the latter by the Ronchesè-Malfatti procedure. To 25 cc. of urine in a 250 cc. flask are added 50 cc. of distilled water, 15 grams of powdered potassium oxalate and a few drops of phenolphthalein.[3] The mixture is then titrated with N/10 NaOH[4] until a permanent pink color makes its appearance. The total acidity is expressed in terms of the volume of standard alkali necessary to neutralize the entire twenty-four specimen of urine. Five cubic centimeters of commercial formalin, which have been neutralized to phenolphthalein, are now added, and the solution again titrated with the standard alkali until the previous pink color is again obtained. By multiplying the number of cubic centimeters thus employed by 0.0017, the grams of ammonia in 25 cc. of urine are learned; and from this the total ammonia output for the twenty-four hours may be estimated.

1. 100 grams of sodium acetate and 100 cc. of 30 per cent. acetic acid to the liter.

2. Dissolve 44.8 grams of uranium nitrate in 900 cc. of distilled water. By titrating this solution with a standard phosphate solution (14.7 grams $HNaNH_4PO_4.4H_2O$ to a liter) the amount of water to be added to the remainder of the uranium solution so that one cc. will be equivalent to the 0.005 grams of P_2O_5 can be calculated.

3. One gram of phenolphthalein dissolved in 100 cc. of 95 per cent. alcohol.

4. For a simple method of preparing standard acids and alkali see footnote Chapter I, p. 6.

If the ammonia nitrogen is desired 0.0014 may be substituted for 0.0017.

Ordinarily the above mentioned method for determining ammonia gives sufficiently reliable data. However, the results are slightly high owing to the presence of amino acids and for this reason when more accurate data are required, it may be advisable to employ the method of Folin.

An accurately measured volume of urine, 10 or 20 cc., is placed in a tall cylinder and after the addition of about one gram of sodium carbonate and 5 to 10 cc. of crude petroleum, the ammonia is aspirated into a bottle containing a definite amount, 10 or 20 cc., of N/10 sulphuric acid and a few drops of congo red[1] as indicator. When the larger volume of urine has been used, it is advisable to employ the Folin absorption tube. In either case sufficient distilled water must be added to completely immerse the absorption tube in the N/10 acid. To exclude any error from ammonia in the air, the air current is first made to pass through dilute sulphuric acid. For 20 cc. of urine, about one and one-half hours will transfer the ammonia to the N/10 acid, while with 10 cc., one-half hour will suffice. Compressed air may be employed in place of the suction. When the operation is completed, the acid not used is titrated with N/10 alkali, the difference being due to the ammonia of the urine. By multiplying this figure by 0.0014, the grams ammonia nitrogen in the urine employed will be found and from this the 24 hour elimination may be calculated.

8. *Total Nitrogen*[2] (*Kjeldahl Method*).—Place 5 cc. of urine in a

1. 0.5 gram of congo red in a mixture of 90 cc. of distilled water and 10 cc. of 95 per cent. alcohol.

2. Folin and his coworkers (*Jour. Biol. Chem.*, 1912, XI, p. 493 *et seq.*) have recently suggested microchemical methods for the estimation of total nitrogen, urea and ammonia, by which the small amounts of nitrogen obtained are ultimately estimated colorimetrically as ammonia by means of Nessler's reagent. There are certain mechanical and chemical difficulties in connection with these methods, however, which render them less available in the examination of urine for scientific and clinical purposes, than the older methods here described. As indicated in the chapter on body fluids, Folin and Denis have demonstrated the great value of these methods in the examination of blood, etc. where the delicacy of the older methods is insufficient.

If greater simplicity is desired for urine, the methods for total nitrogen and urea here described, may be modified by employing 1 or 2 cc. of urine and smaller pieces of apparatus.

Kjeldahl flask, add 20 cc. of concentrated sulphuric acid and a spoonful (2 grams) of potassium sulphate and boil the mixture in the digestion rack until it is entirely colorless. Allow the flask to cool and dilute the contents with about 200 cc. of tap water. Add a little more of a saturated NaOH solution than is necessary to neutralize the sulphuric acid (about 40 cc.) By means of a safety-tube connect the flask with a condenser so arranged that the delivery-tube passes into a vessel containing a known volume (50 cc.) of N/10 sulphuric acid, using care that the end of the delivery tube reaches beneath the surface of the fluid. It is now distilled until about three-fourths of the solution has passed over. Titrate the unused N/10 sulphuric acid by means of N/10 sodium hydroxide using congo red as indicator. One cc. of N/10 sulphuric acid is the equivalent of 0.0014 gram nitrogen. After ascertaining the amount of N/10 sulphuric acid neutralized by the distilled ammonia, calculate the amount of nitrogen in 5 cc. of urine and then in the 24 hour specimen.

9. *Urea.*—

a. *Benedict's Method.*—Five cc. of urine are introduced into a rather wide test-tube, about three grams of potassium bisulphate and one gram of zinc sulphate added, a small quantity of powdered pumice and a bit of paraffin are introduced and the mixture boiled to dryness in a paraffin bath at 130°C and finally heated for one hour at 160–3° C. The residue is then rinsed into a Kjeldahl flask with hot water, 15–20 cc. of 10 per cent. NaOH added and the ammonia is distilled as in the Kjeldahl method. Make correction for the amount of ammonia nitrogen originally present in the urine and calculate the 24 hour elimination of urea nitrogen and of urea.[1]

b. Hypobromite Method.—The instrument which is ordinarily employed in the clinical laboratory with the hypobromite solution is the Doremus ureometer. We have taken occasion to compare the results obtained with several of these instruments as ordinarily graduated, with figures obtained with Benedict's method and also with figures obtained with the hypobromite method employing a gas burette. The hypobromite method when properly carried out with a gas burette gives values which are very close to those with Benedict's method and suffi-

1. The amount of urea excreted may be estimated by multiplying the urea-nitrogen elimination by 2.14.

ciently accurate for clinical purposes, while the Doremus instruments which we have used have given results 20–45 per cent. below the actual values. Obviously such data are valueless. A Lunge or Schiff gas burette may be very conveniently employed in measuring the amount of nitrogen gas evolved by the hypobromite solution, but where these are not available, an ordinary burette may be used by inverting and partly immersing it in water in a tall cylinder. The upper end of the burette is then connected with a bottle of 100 cc. capacity containing about 25 cc. of the hypobromite solution and a small test tube containing two cc. of urine. The rubber stopper is tightly inserted and the burette reading recorded. The bottle is now inclined and the urine allowed to mix with the hypobromite solution.[1] The bottle is shaken at intervals for several minutes, the burette is raised until the water level is the same both inside and outside the burette and the reading then taken. If the tables of Simon and Regnard[2] are at hand the value in grams may be read off directly. If not the following formula may be used:

$$w = \frac{v\,(p-T)}{354.5 \times 760\,(1 + 0.003665\,t)}$$

in which w = weight of urea in grams; v = observed volume of N gas; p = barometric pressure in mm. of mercury; T = tension of aqueous vapor for temperature t; and t = temperature C. The values of the T for the ordinary temperatures are:

Temperature	Tension in mm.
20° C	17.396
21° C	18.505
22° C	19.675
23° C	20.909
24° C	22.211
25° C	23.582

It should be remembered that the hypobromite solution decomposes not only urea, but also any free ammonia and to a con-

1. The solution is made as needed by mixing one part each of two separate solutions with three parts of water. Solution (a) contains 12.5 grams of sodium bromide and 12.5 grams of bromine in 100 cc. of water; and solution (b) is a 22.5 per cent. solution of sodium hydroxide.

2. See Wood: Chemical and Microscopical Diagnosis, 3rd. Edit., 1911, p. 476.

siderable extent all other nitrogenous substances. With a low urea and a high ammonia content this might lead to a very considerable error.

10. *Uric Acid (Folin Method)*[1].—Place 300 cc. of urine in a tall cylinder and add 75 cc. of the Folin-Shaffer reagent.[2] Filter, transfer *two* 125 cc. portions (to serve as duplicates) to two beakers, add five cc. of concentrated ammonium hydroxide and allow the mixture to stand 24 hours. The precipitated ammonium urate is then transferred quantitatively to a hard filter and washed with 10 per cent. ammonium sulphate solution. After removing the filter paper from the funnel and opening it up, the precipitate is washed with about 100 cc. of water back into the same beaker. To this 15 cc. of concentrated sulphuric acid are added and the mixture immediately titrated with N/20 potassium permanganate[3] until the first tinge of pink color extends throughout the fluid after the addition of two drops of the permanganate solution. The average of these two titrations multiplied by 3.75 plus a correction of 3.0 mg. for the solubility of the ammonium urate, gives the mgms. of uric acid in 100 cc. of urine. From this, the calculation for the 24 hours may easily be made.

11. *Creatinine (Folin Method)*.—Pour a little N/2 potassium bichromate[4] into one of the two cylinders of the colorimeter (Duboscq's) and carefully adjust the depth of the solution to the 8 mm. mark. Place 10 cc. of urine in a 500 cc. volumetric flask, add 15 cc. of a saturated solution of picric acid and five cc. of a 10 per cent. solution of sodium hydroxide. Shake thoroughly and allow the mixture to stand for five minutes. At the end of this interval the contents of the 500 cc. flask are diluted to the 500 cc. mark with tap water, thoroughly mixed and a portion poured into the empty cylinder of the colorimeter. A number of readings are

1. Folin and Macallum (*Jour. Biol. Chem.*, 1912, XIII, p. 363) have recently suggested a simple colorimetric method for the determination of uric acid in urine. For several reasons the method here described is to be preferred for urine, although, on account of its delicacy, this new method is the only one available for the determination of uric acid in the blood. (See Chapter X.)

2. 500 grams of ammonium sulphate, five grams uranium acetate and 60 cc. of 10 per cent. acetic acid in 650 cc. of distilled water.

3. 1.581 grams of potassium permanganate dissolved in one liter of distilled water.

4. 24.55 grams to the liter.

taken immediately. 8.1 divided by the reading obtained multiplied by the total volume of urine will give the milligrams of creatinine in the 24 hour specimen.

12. *Creatine* (*Folin-Benedict and Myers Method*).—Pipette two 10 cc. portions of the urine into two 100 cc. Elenmeyer flasks, add 10 cc. of approximately normal hydrochloric acid[1] to each and heat in the autoclave at twenty pounds for one-half hour. This converts any creatine to creatinine. At the end of the interval remove the flasks and allow them to cool. Determine the creatinine plus creatine as above, employing in this case 10 cc. of the 10 per cent. alkali to overcome the increased acidity. The difference between the preformed and the total creatinine gives creatine in terms of creatinine. By multiplying this value by 1.16 the weight of the creatine may be obtained.

1. Prepared by diluting 100 cc. of concentrated hydrochloric acid to one liter with distilled water.

CHAPTER IV.

ALBUMINURIA.

The presence in the urine of protein substances may conveniently be considered under the heading of disorders of protein metabolism. Practically all of the proteins present in the body, including their cleavage products have been found to appear in the urine under pathological conditions. Among these are serum albumin and serum globulin, hemoglobin, fibrinogen, nucleoprotein, glucoprotein, Bence Jones' protein, proteoses, peptones and amino acids, notably tyrosine, leucine, cystine, glycine, etc. As previously mentioned, the facts at our disposal indicate that the end products of proteolytic digestion are the amino acids, and further, that they are absorbed into the blood as such. Just where or how they are employed for the repair of body tissue or for the formation of the circulating proteins of the blood, has not been ascertained. The presence of a protein substance in urine may be due to one of several factors, to the increased permeability of the kidney, to the presence in the blood of foreign protein material, or to normal material in abnormal amounts.

Albumin.—In referring to the presence of albumin in the urine clinically, the term includes both the blood proteins, serum globulin as well as serum albumin, though the latter is nearly always in excess. The ratio which exists between the paraglobulin and the albumin of the blood in man is about 1 to 1.5, while in the urine, the ratio generally falls between this point and 1 to 2.3, the amount of the globulin being the variable factor. In contracted kidney and in chronic passive congestion, the quotient may be found between 2.8 and 5.3, though in cases of nephritis in which there are extensive lesions in the renal epithelium, it may be lowered, and in amyloid disease it may be below one. From the foregoing, it is apparent that albumin and globulin nearly always exist together in the urine, though the former is, as a rule, the more abundant. Cases are on record, however, where very large amounts of globulin have been eliminated in the urine.

The more important conditions under which albumin may appear in the urine may be referred to briefly as follows: There are persons, apparently perfectly healthy, who continually, or at intervals, secrete urine in which albumin may easily be demonstrated. The kidneys of these individuals may not necessarily be the site of a true nephritis, though the presence of albumin would indicate some inefficiency.

Under the head of the so-called physiological albuminurias are classed those cases, which, though symptoms may be absent, excrete albumin after cold baths, violent physical exertion, or following the taking of an abundance of food, especially many raw eggs. In this latter case it has been shown that a portion of the albumin may be unchanged egg albumin. Recently, the elimination of albumin following such severe athletic contests as basket ball has been shown not to be an unusual occurrence.[1]

Albuminuria due to circulatory disturbances may follow changes in the kidney resulting from the altered blood pressure, which is frequently observed in severe and uncompensated heart lesions. In some way, not clearly understood, the retarded circulation seems to injure the renal cells. The quantity of albumin is usually small and a few hyaline casts may be observed.

Injury to the renal cells produced by toxic substances, whether mineral or organic poisons, may result in albuminuria. Among such poisons are arsenic and uranium compound, chromates, cantharides, ether, etc.

Albuminuria occurs in many of the well-known febrile conditions, *i.e.*, typhoid fever, due to degenerative changes in the kidney epithelium, possibly produced by the toxins formed. In the severer cases this may develop into a true nephritis with much albumin and many casts.

The albuminuria of nephritis[2] is of particular interest. In *acute nephritis* the elimination of large amounts of albumin is a constant and most important symptom, the amount eliminated being in general in proportion to the severity of the condition. Usually about five to eight grams of albumin are eliminated in

1. Fischer: Nephritis, New York, 1912, pp. 47 and 182.

2. Barker: *Amer. Jour. Med. Sci.*, 1913, CXLV, pp. 42--68, has recently given a most interesting and comprehensive discussion of the commoner forms of renal disease, with special reference to the knowledge of them most useful at present to the general practitioner.

twenty-four hours, though on rare occasions, the amount may reach 20 grams. The elimination of albumin is likewise a constant association of *chronic parenchymatous nephritis*, the quantity eliminated generally amounting to five grams a day, though it may exceed that of the acute variety and reach to 15 to 30 grams daily. In the majority of cases of *chronic interstitial nephritis*, on the other hand, the elimination of albumin is slight, rarely amounting to more than two to five grams per day. In fact it is not unusual to meet with an apparent absence of albumin, as indicated by the less delicate tests. Obviously a careful examination for albumin and casts is here of very great diagnostic importance. The amount of albumin observed in *amyloid degeneration* is quite comparable to chronic interstitial nephritis. An entire absence of albumin is less frequent, however, the quantity eliminated generally amounting to one to two grams per day, though as pointed out above, large amounts of serum globulin are sometimes excreted.

Nucleoprotein.—Nucleoprotein occurs in normal urine only in minute traces, but in larger amounts in the urine of the new born, after over exertion, in inflammation of the mucous membrane of any part of the urinary tract, in leukemia, in chronic parenchymatous nephritis, and very abundantly during jaundice. Clinically, its presence in the urine in increased amounts is simply an indication of some irritation along the urinary tract.

Bence Jones' Protein.—In association with multiple myeloma of the bone, a peculiar protein substance has been observed in the urine in 80 per cent. of the cases and is apparently pathognomonic of the disease.[1]

Proteoses.—Proteoses (albumoses) are present in traces in the urine in most febrile diseases and in quite a variety of other conditions, notably those in which a septic condition exists in some part of the body. They have been observed in ulceration of the intestine, in typhoid and in dysentery, occasionally in malignant growths, probably due to ulceration, in abscess of the liver, in empyema, and in phosphorus poisoning. Their presence in the urine in meningitis is supposed to be in favor of a suppurative process, and against a tubercular one.

Amino Acids.—Glycocoll is the one amino acid which appears to be presenti n normal urine in small amounts, though traces

1. For literature consult Rosenbloom: *Biochem. Bul.*, 1911, I, p. 161.

of other amino acids probably exist. In certain severe organic diseases of the liver, as acute yellow atrophy, chloroform necrosis, phosphorus poisoning, eclampsia, and occasionally in severe infections and diabetic coma, different amino acids appear in the urine, leucine, tyrosine, and cystine being the most important. Whether the presence of these acids in the urine is due to the inability on the part of the body to utilize them or to a failure of the deaminization reaction, which possibly takes place in the liver, is not clear. In a very recent paper, Levene and Van Slyke[1] report a series of determinations of the amino acid content of normal and pathological urines. Cases of arithritis, gout, carcinoma of the breast and nephritis were observed in which there was a notable increase in the elimination of nitrogen in this form.

In two very interesting but rare congenital anomalies of protein metabolism, cystinuria and alkaptonuria, there appears to exist a faulty metabolism of certain individual amino acids. In the case of cystinuria, the sulphur containing amino acid, cystine, is unutilized and is eliminated in the urine, the amount excreted per day varying between a few centigrams and a gram. In the case of alkaptonuria, a faulty metabolism of the phenyl amino acids, tyrosine and phenylalanine, is observed. The body appears to be unable to carry the transformations of these acids beyond the homogentisic acid (dioxyphenylacetic acid) stage and this compound then appears in the urine. Neither of these conditions, however, is connected with any clinical symptoms.

Metabolism in Renal Disease and the Permeability of the Kidney.—Eccentric and unaccountable variations in the elimination of the urinary nitrogen are observed in renal diseases, a decreased elimination being followed by a compensatory increase. In acute nephritis, however, it is usual to find a decreased elimination of nitrogen and especially of its chief component, urea, though there are periods in the course of a nephritis in which the urea excretion is perfectly normal. The elimination of ammonia and uric acid is said to be normal. As regards the elimination of sodium chloride, its retention appears to go hand in hand with that of urea, the greatest retention being likewise observed in acute nephritis. The decreased excretion of water is coincident with the formation of edema and the retention of

1. Levene and Van Slyke: *Jour. Biol. Chem.*, 1912, XII, p. 310.

salt, in which the sodium chloride appears to play a very important part. Whether the salt is retained because of the impermeability of the kidneys, and the water needed to preserve the proper osmotic relations, or the water is taken up by the hydrophylic colloids of the tissues and the salt subsequently retained to maintain the normal osmotic pressure of the body fluids, is not clear.

As regards the nitrogen intake of individuals suffering from nephritis, it seems to be advisable as shown by von Noorden[1], to reduce the nitrogen intake to the lowest possible level in acute nephritis, as here, and in acute inflammatory exacerbations of chronic renal disease, a large protein intake undoubtedly exercises an injurious effect on the albuminuria. Even a milk diet is too rich. In the chronic forms, the protein intake should not be reduced below 80 to 90 grams per day; otherwise, the patients become progressively weaker. Nitrogen equilibrium may be maintained, however, even with considerable loss of protein in the urine, if the nitrogen intake is high enough. The extended observations of von Noorden would indicate that in interstitial nephritis—the most frequent and important of all forms of kidney disease—the degree of albuminuria is in no way influenced by the protein intake.

Though the estimation of urea has long been employed clinically as a method of estimating the efficiency of the kidney, it is questionable whether it is ever of much value from this standpoint, even when the urea is accurately determined and the nitrogen content of the diet known. A test, which has met with considerable favor, has recently been described by Rowntree and Geraghty (see below). This is a permeation test in which a known amount of phenolsulphonephthalein is given, and the ability of the kidney to eliminate the drug ascertained. The test appears to reveal the degree of functional derangement in nephritis, whether of the acute or chronic variety. It has also been of value in diagnosing impending uremia and distinguishing uremia from conditions simulating it.

LABORATORY PROCEDURES.

Of the various tests which have been employed for the detection of protein substances in urine, the greater number involve

1. von Noorden: Metabolism and Practical Medicine, Eng. Ed., 1907, II, p. 477; also POST-GRADUATE, 1913, XXVIII, p. 3.

precipitation reactions. In some of these the protein is precipitated because of its insolubility in the reagent employed; in others, by the action of the reagent to form an insoluble compound with the protein, as in the case of the alkaloidal reagents. In performing all these tests it is essential that the urine should be perfectly clear. If it is not clear, filtration through filter paper will usually suffice for clarification, but in case this is ineffective, the urine may be shaken with powdered magnesia, or kaolin and again filtered.

1. *Heller's Test.*—When properly applied, this test is one of the most satisfactory general tests we possess. About one cc. of pure nitric acid is placed in a small test tube. By means of a pipette having a small rubber bulb at one end and a ragged non-tapering edge at the other, an equal amount of urine may be allowed to flow down the side of the tube without inclining or removing from the rack. By this procedure, a perfect stratification may be obtained with great rapidity. In the presence of albumin a white zone of precipitated albumin will be observed at the point of juncture of the two liquids. If the albumin is present in very small amount, the white zone may not form until the tube has been allowed to stand several minutes. With concentrated urines, uric acid or urea may occasionally cause confusion to the inexperienced, due to the precipitation of uric acid and urates, or the formation of urea nitrate. Simply diluting the urine will remove these difficulties. The fine ring which appears in the clear urine above and separated from the albuminous ring is generally ascribed to the presence of urates though certain investigators regard it of a protein nature. After the administration of certain drugs, a white precipitate of resin acids may form at the contact of the two fluids. This being the case the ring will dissolve in alcohol, whereas the albumin ring will not dissolve. Biliary urine reacts with nitric acid containing a little nitrous acid to give the play of colors referable to the action of nitric acid upon bilirubin.

2. *Robert's Test.*—This test is carried out in the same manner as Heller's test, except that Robert's reagent[1] is substituted for the nitric acid of the previous test. With this test colored rings do not form and the test is slightly more sensitive than

1. The reagent is prepared by mixing five parts of saturated magnesium sulphate and one part conc. nitric acid.

Heller's, but is subject to the same disadvantage in that nucleoprotein and mucin are precipitated.

3. *Heat Test.*—From 10 to 15 cc. of clear urine are placed in a test tube, and the reaction of the urine tested. If it is not faintly acid, it is rendered so with a few drops of very dilute (2 per cent.) acetic acid. The upper part of the tube is brought to the boiling point, when in the presence of albumin, a white cloud will be observed, in comparison with the control portion of the tube below. The cloud may in part be due to nucleoprotein and mucin, but the addition of one sixth volume of saturated sodium chloride and 5 drops of 50 per cent. acetic acid, with the subsequent boiling of the upper part of the tube will serve to distinguish it from these two proteins, as in this case the nucleoprotein and mucin are not precipitated. The test carefully applied in this way is very reliable.

4. *Sulphosalicylic Acid and Trichloracetic Acid Tests.*—These two tests may be mentioned together because of their great delicacy and the general similarity of the reactions. The sulphosalicylic acid may be used in the form of a 20 per cent. aqueous solution, and the trichloracetic as a saturated aqueous solution. The clear urine is stratified upon the solutions as in Heller's test. Proteoses (albumoses) are precipitated, but dissolve on warming, and reappear on cooling. In concentrated urines, urates may be precipitated with the trichloracetic acid, but this can be avoided by the dilution of the urine. In the case of sulphosalicylic acid, uric acid and the resins are not precipitated. If a delicate test is desired these reagents are particularly valuable, as for example when tube casts have been observed, though previous tests for albumin have been negative.

5. *Quantitative Estimation of Protein.*—It cannot be said that we possess any very satisfactory clinical method for the estimation of protein in urine. Accurate results may be obtained by coagulating the protein, filtering through a weighed Gooch crucible with asbestos or glass wool mat, drying and again weighing, but this method can hardly be applied in ordinary routine work. The method of Esbach with the original picric acid solution yields fairly satisfactory results. In the hands of the authors Tsuchiya's phosphotungstic acid reagent has not been found as accurate as the original picric acid solution when

employed in the Esbach albuminometer. Still another method which yields fairly reliable results under properly controlled conditions is the method of Purdy, in which the albumin is precipitated by acetic acid and potassium ferrocyanide and the precipitate thrown down in a graduated centrifuge tube. It is very difficult, however, to keep a centrifuge in a condition that it will maintain the necessary 1500 revolutions per minute.

a. *Esbach's Method.*—Fill the albuminometer to the mark "U" with urine acidified if necessary with a few drops of dilute acetic acid and add Esbach's reagent[1] to "R." The specific gravity of the urine should not exceed 1.008, the proper density being obtained by accurate dilution with water. Stopper the tube, invert it slowly several times in order to insure thorough mixing of the fluids, and set aside for twenty-four hours at a temperature of about 15° C. The height of the precipitate on the scale indicates directly the number of grams of dry protein in a liter of the urine. In case it is desirable to ascertain the quantity of albumin at once, employ Kwilecki's modification as follows: add 10 drops of 10 per cent. ferric chloride to the acid urine before introducing the Esbach reagent, warm the tube and contents in a water bath at 72° C. for five minutes and take the reading.

If it is desired to employ Tsuchiya's phosphotungstic acid reagent,[2] this may be used in the same tube and same way as the Esbach reagent.

b. *Purdy's Method.*—Ten cc. of clear urine are placed in a 15 cc. graduated centrifuge tube, 3 cc. of 10 per cent. potassium ferrocyanide and 2 cc. 50 per cent. acetic acid added. The urine and solutions are mixed, the tube set aside for 10 minutes to allow precipitation of the albumin and then centrifugalized for exactly three minutes at 1500 revolutions per minute, in an instrument with a radius, including the tubes, of just 6¾ inches. The tube is then removed and the grams protein per liter read off from the following table compiled from Purdy.[3] When the amount of protein is large the urine should be accurately diluted.

1. The reagent is composed of 10 grams of picric acid and 20 grams of citric acid dissolved in 1000 cc. of distilled water.

2. Tsuchiya's reagent consists of 1.5 grams of phosphotungstic acid dissolved in 5 cc. conc. hydrochloric acid and 95 cc. of alcohol.

3. Purdy: Practical Urinalysis and Urinary Diagnosis, 6th edit., Philadelphia, 1901, p. 80.

Volume of precipitate in graduated tube.	Dry weight of protein to liter	Volume of precipitate in graduated tube	Dry weight of protein to liter
cc.	grams	cc.	grams
0.25	0.5	2.75	5.7
0.5	1.0	3.0	6.3
0.75	1.6	3 25	6.8
1.0	2.1	3.50	7.3
1.25	2.6	3.75	7.8
1.5	3.1	4.0	8.3
1.75	3.6	4.25	8.9
2.00	4.2	4.50	9.4
2.25	4.7	4.75	9.9
2.50	5.2	5.0	10.4

Detection of Other Protein Substances in Urine.—It is occasionally of importance to examine urine for other protein substances, aside from albumin and globulin. Among such are nucleoprotein, hemoglobin, "Bence Jones" protein, proteoses and amino acids, the Bence Jones protein being very rare, and associated with multiple myeloma of the bone.

5. *Nucleoprotein.*—Nucleoprotein cannot be positively identified in urine, especially in the presence of other protein substances without considerable difficulty. If urine diluted about three times shows a turbidity when made strongly acid with acetic acid, it indicates the presence of nucleoprotein. If albumin is also present this should previously be removed by boiling and filtering.

Ott's test has been supposed to demonstrate nucleoprotein. A few cc. of urine are mixed with an equal volume of saturated salt solution and Almen's reagent[1] slowly added. A bulky precipitate appears in the presence of nucleoprotein.

6. *Detection of Proteoses—Method of Bang.*—Ten cc. of urine are saturated with ammonium sulphate with the aid of heat (about 10 grams required) and then brought to boiling. The precipitate is thrown down by centrifuging, then rubbed up in a mortar with 96 per cent. alcohol to remove urobilin. The alcohol is poured off and the residue treated with distilled water, warmed and filtered. The filtrate contains the proteose which will be shown by the biuret reaction, *i.e.*, the addition of strong

1. Prepared by dissolving five grams of tannin in 240 cc. of 50 per cent. alcohol and add 10 cc. of 25 per cent. acetic acid.

caustic alkali followed by a few drops of very dilute copper sulphate, resulting in the production of a pink color in the presence of proteoses.

7. *Amino Acids.*—Very recently Benedict and Murlin[1] in a preliminary note have suggested a simple modification of the Henriques-Sörensen formol titration for amino acids by which they may be titrated directly. The ammonia and certain of the other urinary compounds except the urea are removed from the urine by precipitation with 10 per cent. phosphotungstic acid in a strongly acid solution, allowing 24 hours for sedimentation. The excess of phosphotungstic acid is now removed by means of tribasic lead acetate and litharge. The amino acids are now titrated in the filtrate from the above, after the removal of the excess of lead, by the formalin titration method as previously described for ammonia.[2]

8. *Estimation of Renal Efficiency*[3] *with Phenolsulphonephthalein.*—Rowntree and Geraghty[4] have recently proposed a simple permeation test which appears to be very efficient. The test: 20 to 30 minutes before administering the drug, the patient is given 200 to 400 cc. of water to insure copious urinary secretion. Under aseptic precautions, a catheter is introduced into the bladder and the latter completely emptied. Noting the time, one cc. of a carefully prepared solution of phenolsulphone-

1. Benedict and Murlin: *Proc. Soc. Exp. Biol. Med.*, 1912, IX, p. 109.

2. Consult Chapter III, page 43.

3. Mention should also be made of the very interesting and promising work of Schlayer and his pupils in Romberg's Clinic (see papers in recent vols. of *Deutsch. Arch. f. klin. Med.*) From the results of their experimental and clinical work these investigators are of the opinion that sodium chloride and potassium iodide are excreted by the epithelium of the renal tubules, and that lactose and water are excreted by the glomeruli. They believe that in this way one may differentiate renal diseases with predominantly tubular lesions from those with predominantly vascular lesions. For discussion see Schlayer: *Medizinischen Klinik*, 1912, VIII; Eppinger and Barrenscheen: *Wien. klin. Wochenschr.*, 1912, LXII; also Baright: POST-GRADUATE, 1913, XXVIII, p. 317, who gives simplified technique of tests. Rowntree, Fitz and Geraghty (*Arch. Int. Med.*, 1813, XI, pp. 121 and 258) in recent experiments confirm the value of lactose as a very delicate diagnostic test, though they do not regard the sodium chloride and potassium iodide tests with especial favor.

4. Rowntree and Geraghty: *Jour. Pharmacol. Exper. Therap.*, 1910, I, p. 579; *Jour. Amer. Med. Assoc.*, 1911, Vol. LVII, p. 811; *Arch. Int. Med.*, 1912, IX, p. 284.

phthalein, containing 6 mgms. to the cc., (may be obtained already prepared in the form of ampoules) is carefully administered subcutaneously or preferably intramuscularly in the lumbar muscles by means of an accurately graduated syringe. The urine is now allowed to drain into a test-tube in which has been placed a drop of 25 per cent. sodium hydroxide and the time of the appearance of the first pinkish tinge noted. A rough estimate of the time of appearance can be made by having the patient void urine at frequent intervals without the use of a catheter. Where the catheter has been employed and there is no urinary obstruction, the catheter is withdrawn at the time of the appearance of the drug in the urine, and the patient is instructed to void into one receptacle at the end of one hour and into another receptacle at the end of two hours. If it is desired to distinguish between the efficiency of the two kidneys, the urine may be collected separately from each kidney by catheterizing the ureters. The drug normally appears in the urine in five to ten minutes, 38 to 60 per cent. being eliminated the first hour and 60 to 85 per cent. during the two hours. The test is of particular value in determining the degree of functional derangement in nephritis, whether of the acute or chronic variety, also in uremia, etc. The percentage elimination is estimated by treating the urine of the two periods with sufficiently strong sodium hydroxide to produce the maximum red color and each diluted to 1000 cc., if the depth of the color will allow. This is then compared in a Duboscq colorimeter with a standard solution containing 3 mgms. of the drug to the liter, the control prism being set at 10 mm. (Rowntree and Geraghty now employ the cheaper Hellige instrument). With a colorimeter, the determination of the percentage elimination is very simple and rapid. A series of ten test tubes containing solutions of phenolsulphonephthalein of different known concentration may be prepared, the equivalents of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 per cent. of the injected drug, with which the drug excreted in the urine, when diluted as above, may be measured. These standard solutions will keep for a considerable length of time if an excess of alkali has been added and the test tubes stoppered and sealed with paraffin. By this method the quantitative excretion of the drug may be approximately ascertained, probably within 5 per cent. of the correct value, and will obviate the need for a colorimeter.

CHAPTER V.

Glucosuria and Other Types of Mellituria.

The elimination of appreciable quantities of sugar in the urine is evidence of some inefficiency of carbohydrate metabolism or indiscretion of diet.[1] The metabolic defect may be traced to lesions of certain organs, *e.g.*, the pancreas, or to abnormal development of other glands such as the thyroids or hypophysis, while not infrequently there is no obvious cause. Sugars other than glucose may appear in the urine. Thus in certain instances, arabinose, levulose, galactose, lactose, maltose and sucrose may be eliminated; and for the condition characterized by the presence in the urine of a sugar without specification, the term "mellituria" is appropriate.

Since, as stated, most types of mellituria are results of deviations from physiological functions, consideration of these conditions will be preceded by a brief review of the processes of carbohydrate metabolism occurring normally. The blood of a normal individual contains from .07 to .11 per cent. of glucose. Ordinarily the concentration of the latter does not exceed these limits, however, great or small the carbohydrate intake, and is essentially independent of the fuel requirements of the body. Should the mechanism, by which this constancy is maintained, become defective, glucose will accumulate in the blood, which condition of hyperglucemia is the immediate cause of glucosuria.

Usually the body is supplied with glucose at intervals during the day, the amount ingested at any one period being in excess of the immediate fuel requirements. By means of an enzymatic process this excess is converted into and stored as glycogen in the liver (glycogenesis), the reverse action, (glycogenolysis) occurring as more glucose is needed for combustion. The storage capacity of the liver for glycogen is approximately 150 to 200 grams; and should this limit be exceeded, the remaining sugar would be retained and stored as glycogen in the muscles.

1. The urine of a normal individual may contain .01 to .06 per cent. of glucose. Such small quantities, however, cannot be detected by the usual clinical methods.

The concentration of glycogen in the muscle tissue may reach two per cent. and in special cases four per cent., corresponding to sufficient carbohydrate to maintain body heat for two to four days. If the carbohydrate intake is so great that the storage capacity of both the liver and muscles is overtaxed, the glucose not converted to glycogen is further prevented from giving rise to hyperglucemia and glucosuria by being transformed into fat. A large amount of sugar can be stored in this manner. There is, likewise, a limit to fat formation and should this point be reached, still another means is available by which the excess of blood sugar may be removed from the circulation—elimination through the kidneys. When all the storage depots become loaded to their full capacity, the sugar, in excess of that required to meet the fuel needs of the body and to maintain essentially constant the sugar concentration of the blood, is excreted in the urine. Any one or more of these regulatory mechanisms may become defective, allowing an excess of sugar to accumulate in the blood and thus give rise to glucosuria. Examples of such defects follow.

Glucose may be absorbed from the alimentary tract and be supplied to the liver more rapidly than it can be converted into glycogen. The sudden accumulation of sugar in the blood would lead to glucosuria—"alimentary glucosuria." Normally an administration upon the empty stomach of 100-200 grams of glucose will be tolerated, and this serves as a test of the power of glycogenesis. Glucosuria following the ingestion of even very large amounts of starch—"glucosuria *ex amylo*"—is practically never observed in the healthy person. There are individuals in whom the function of glycogenesis is only slightly impaired, while in an advanced case of diabetes it is almost completely destroyed. If the other storage places are efficient and if the power of burning sugar is not impaired, there may be only a transient glucosuria as a result of the defective glycogenesis. This condition is presumably to be attributed to the absence or insufficiency of an active glycogenetic enzyme, although it may be due primarily to disease of the pancreas. It is interesting to note that even in advanced cases of organic disease of the liver the function of the formation of glycogen from glucose is retained, although frequently in these conditions the liver is unable to properly utilize levulose and hence, ad-

ministration of this sugar often gives rise to levulosuria. Glucosuria is noted following the ingestion of moderate doses of sugar in gout, obesity, exophthalmic goitre, hypertrophic cirrhosis of the liver, fatty liver, pneumonia, influenza, alcoholism and lead poisoning.

In addition to defective glycogenesis, there may be an exaggeration of the function of glycogenolysis, whereby glycogen is more rapidly transformed into glucose than it is needed for combustion and also in excess of the capacity of the muscular system to reconvert the liberated glucose into glycogen. Consequently an excess of sugar finds its way into the circulation and glucosuria results. This type of glucosuria is of nervous origin and is probably due to stimulation of the liver cells which elaborate the glycogenolytic enzyme. It is possible that the nervous influences bring this about indirectly by primarily stimulating the adrenals (see p. 66.) Experimentally, glucosuria may be evoked in the rabbit by puncturing the floor of the fourth ventricle as Claude Bernard showed many years ago. Frequently glucosuria of this type follows traumatism of the nervous system, and indirect stimulation of any part of the nervous system, if sufficiently strong, may produce glucosuria. Fright or excitement may be associated with the appearance of glucose in the urine.[1] Cases of nervous glucosuria are not usually persistent. Under this head would appear to come the glucosuria resulting from the administration of large doses of certain poisons, such as strychine, morphine, amyl nitrite, prussic acid, ether, chloroform, carbon monoxide, and many others. Underhill[2] has called attention to the respiratory disturbances accompanying the use of some of these compounds, and Henderson and Underhill have pointed out the frequency with which acapnia is associated with glucosuria.[3] They believe that ether glucosuria is due to acapnia, which also is usually the cause of traumatic and emotional glucosurias. Henderson and Underhill further observed that they never found glucosuria in dogs which had been brought quietly into deep ether anesthesia.

1. Cf. Cannon, Shohl and Wright: *Amer. Jour. Physiol.*, 1911, XXIX, p. 280.

2. Underhill: *Jour. Biol. Chem.*, 1905, I, p. 124.

3. Henderson and Underhill: *Amer. Jour. Physiol.*, 1911, XXVIII, p. 275.

Glycogenes's may be reduced and glycogenolysis may be excessive in the muscles as well as in the liver. Either of these conditions would tend to produce hyperglucemia and glucosuria. Both of these abnormal relations between sugar and glycogen in the muscles are present in diabetes.

It has been stated that a large amount of sugar can be transformed into and stored as fat, but that there is a limit to this fat formation. It is apparent that very obese individuals are living close to this limit, since a slight excess of carbohydrate in the diet frequently gives rise to glucosuria. On the other hand there are individuals, who have very great powers of combustion, and they store fat only with considerable difficulty. Advanced cases of diabetes have lost not only the power of burning sugar but also the ability to store fat. Occasionally there are encountered diabetics who become very obese, such individuals having apparently retained the function of forming fat although their sugar burning powers are reduced to a minimum.

In addition to the above mentioned types of glucosuria there are certain others less well defined, such as the glucosuria of adolescence and the glucosuria associated with certain skin diseases.

Hyperglucemia is the immediate cause of all the instances of glucosuria just discussed. In a condition of hyperglucemia more sugar is present in the blood than can be retained by the kidneys, and hence glucosuria results. The level of renal retention for sugar may be raised or lowered. In certain cases of diabetes, especially when complicated with nephritis, this level may be raised, thus permitting a marked hyperglucemia without a correspondingly strong glucosuria. On the other hand, there have been reported cases of glucosuria, which could most readily be explained by assuming that the level of renal retention had been lowered.[1] Such cases of "renal glucosuria" are characterized by no hyperglucemia, but, nevertheless, excrete continuously over long periods of time small amounts of sugar; and the condition is apparently uninfluenced by the presence or absence of carbohydrate in the diet. Experimentally renal glucosuria may be induced by injections of phlorhizin. Following the use of this compound there are observed marked hypoglucemia and strong glucosuria. To increased permeability of the kidney,

1. For descriptions of several cases of glucosuria of this type, see Garrod: *Lancet*, March 9, 1912, p. 634.

Underhill and Closson have attributed the hypoglucemia and glucosuria resulting from injections of sodium chloride into the venous circulation of the rabbit.[1]

RELATION OF THE INTERNAL SECRETIONS TO CARBOHYDRATE METABOLISM.[2]

Pancreas.—In diabetes the underlying disturbance is an inability on the part of the muscle cells to burn glucose, and is attributed to an insufficiency or absence of the internal secretion of the pancreas. It is believed that this secretion furnishes something which activates the glucolytic enzyme of the muscles, which by itself is unable to bring about the combustion of sugar. Experiments *in vitro* have not been especially confirmatory. When a mixture of muscle, pancreas and glucose is allowed to incubate, there is indeed a diminution in the glucose content of the mixture, but the presence of oxidation products, *e.g.*, lactic acid and alcohol, cannot be detected.[3] On the contrary, recent studies indicate that the reaction proceeds in the other direction, that is, glucose is transformed into maltose.[3] The view is nevertheless held that in the body, the internal secretion of the pancreas is essential to carbohydrate oxidation; and to the islands of Langerhans has been attributed the function of elaborating this secretion. From the intimate relations of the pancreas to diabetes, one would expect to find distinct lesions of this organ; but these are not always obvious. Moreover, serious but more or less localized diseases of the pancreas are noted, which are not regularly accompanied by glucosuria. Indeed, when it is recalled that a large part of the pancreas may be removed without evoking diabetes, it is not difficult to understand that in such pancreatic diseases a sufficient number of the islands of Langerhans may have remained functionally active. It is the diffuse type of pancreatic disease that is more constantly associated with diabetes.

Thyroids.—Exophthalmic goitre is not infrequently associated with diminished tolerance for carbohydrates or in fact with severe diabetes. This is attributed to an overactivity of the thyroid

1. Underhill and Closson: *Amer. Jour. Physiol.*, 1906, XV, p. 321.

2. For an interesting description and discussion of cases, see Garrod: *Lancet*, March 2 and 9, 1912, p. 557 and 629.

3. Levene and Meyer: *Jour. Biol. Chem.*, 1912, XI., p. 356.

glands whereby an excess of their secretion is formed. Moreover, administration of thyroid preparations to normal individuals likewise leads to glucosuria. The suggestion has been made that the excess of thyroid secretion inhibits the action of the pancreas directly, or indirectly by stimulating the formation of adrenaline, injection of which is known to produce glucosuria. While exophthalmic goitre (hyperthyroidism) is accompanied by a diminished tolerance for carbohydrate, myxedema (hypothyroidism), on the other hand, is usually associated with increased tolerance. Individuals with myxedema are able to utilize quantities of carbohydrate, which would be sufficiently great to cause alimentary glucosuria in the normal person. This increased tolerance for carbohydrate may be lowered to the normal level by the adminstration of thyroid preparations. Although usually diminished tolerance for sugar is a concomitant of hyperthyroidism and increased tolerance is associated with hypothyroidism, there are, nevertheless, cases of exophthalmic goitre, in which glucosuria cannot be demonstrated, and likewise instances where myxedema is accompanied by diabetes. These apparently contradictory findings need not necessarily discredit the view that hyperthyroidism and hypothyroidism are associated respectively with diminished and increased tolerance for carbohydrate. A case of myxedema may be complicated with a diseased pancreas, and the ensuing glucosuria may persist in spite of the diminished activity of the thyroid, which would lead to increased tolerance, were the pancreas normal. Furthermore, it is not impossible that the inactivity of the thyroid would result in hypertrophy of the pituitary glands, and the secretion of the latter, as will be noted below, lowers the tolerance for carbohydrate. The condition of the parathyroids may be a factor of importance, since, according to Eppinger, Falta and Rudinger, their removal leads to diminished tolerance.[1]

Hypophysis.—Just as exophthalmic goitre is due to hyperactivity of the thyroid, so acromegaly is believed to be the result of an overdevelopment of the pituitary body. Acromegaly, like Graves' disease, is accompanied by lowered carbohydrate tolerance or even a true diabetes. Out of 176 cases of acromegaly,

1. Eppinger, Falta and Rudinger: *Zeit. f. klin. Med.*, 1909, LXVII, p. 380.

Borchardt found 35 per cent to be glucosuric.[1] It occasionally happens that the glucosuria accompanying hyperpituitarism ceases for a considerable length of time or even permanently. This has been accounted for by assuming that the disappearance of glucosuria is coincident with the change from a condition of hyperpituitarism to one of hypopituitarism, which is characterized by increased tolerance. An analagous situation is encountered in the case of the thyroid, where exophthalmic goitre is followed by myxedema.

Adrenals.—Injection of adrenaline induces hyperglucemia and glucosuria, but an undoubted instance of glucosuria due to disease of the adrenals is rare. It has been suggested that an excessive formation of adrenaline may be the cause of certain mild types of diabetes of later life; and it has been shown by Cannon, Shohl and Wright[2] that in the case of cats which have been excited by a barking dog, there is an increased concentration of adrenaline in the blood, and this is accompanied by glucosuria. Porges[3] has demonstrated the condition of hypoglucemia following adrenalectomy and in Addison's disease—presumably a case of deficient adrenal secretion. Hypoglucemia has also been demonstrated following the administration of phosphorus[4] and hydrazine,[5] both of which drugs have been assumed to bring about this result by inhibiting the formation of adrenaline. Underhill and Fine have noted hypoglucemia and absence of glucosuria in dogs under the influence of hydrazine even after extirpation of the pancreas.[6] This is in accord with the observations of Eppinger, Falta and Rudinger that in cases of Addison's disease the carbohydrate tolerance is abnormally high.[7]

Glucosuria of Pregnancy.—There are instances in which pregnancy is associated with glucosuria, the latter usually disappearing after parturition. To temporary overactivity of the thyroid or pituitary has been ascribed this type of glucosuria.

1. Borchardt: *Zeit. f. klin. Med.*, 1908, LXVI, p. 332.
2. *Loc. cit.*
3. Porges: *Zeit. f. klin. Med.*, 1909, LXIX, p. 341.
4. Frank and Isaac: *Arch. f. exper Path. u. Pharm.* 1911, LXIV, p. 274.
5. Underhill: *Jour. Biol. Chem.*, 1911, X, p. 159.
6. Underhill and Fine: *Jour. Biol. Chem.*, 1911, X, p. 271.
7. Eppinger, Falta and Rudinger: *Zeit. f. klin., Med.*, 1908, LXVI, p. 1; *ibid*, 1909, LXVII, p. 380.

DIABETES MELLITUS.

Severe diabetes is characterized by a copious excretion of urine, the volume being roughly proportional to the amount of sugar eliminated. An output of three to six liters is commonly observed and in exceptional cases the quantity may reach ten liters or more, the highest elimination recorded being 28 liters. In diabetes, the volume and specific gravity do not present the inverse relationship usually observed in the normal urine. Thus specific gravities of 1.025 to 1.046 may be noted with volumes of two to ten liters. The highest specific gravity recorded is 1.074.

A more detailed consideration should be accorded the subject of diabetes since it is such a fundamental disturbance of carbohydrate metabolism and involves such important changes in the metabolism of protein and fat. Taylor classifies cases of diabetes under three heads, essentially as follows:[1]

a. Cases occurring most frequently before middle life, of relatively rapid onset, and usually terminating fatally. They exhibit all the typical metabolic derangements of diabetes. The cause is difficult to ascertain. Post mortem, lesions of the islands of Langerhans are usually found.

b. Those cases characterized at first merely with an alimentary glucosuria, but which gradually pass into typical diabetes, and, excepting their more chronic nature, differ in no important respect from the first mentioned type of diabetes. Such cases are most frequently observed after middle life. Some of these cases are attended with all the metabolic defects of diabetes, although a large number retain at least the function of fat formation for a considerable length of time.

c. Cases of diabetes resulting apparently from some previous disease, *e.g.*, gout, arteriosclerosis, obesity, or cirrhosis of the liver. A small percentage of the cases of this class do develop into typical diabetes, but this is not true of the majority. Glucosuria may persist for years with but few of the metabolic abnormalities of diabetes.

It is among cases of the two last types that one fails to obtain undoubted evidence of pancreatic lesions.

The principal defect of metabolism in the diabetic is the in-

1. A. E. Taylor: " Digestion and Metabolism," Philadelphia and New York, 1912, p. 291.

ability to burn sugar. As already described, this is believed to be due to an inefficiency of the pancreas. The results of extirpation of the pancreas closely parallel the conditions found in human diabetes. Glycogenesis is reduced and glycogenolysis is excessive in both the liver and muscular tissue; oxidation of sugar is reduced to a minimum; there is little if any fat formation, and the combustion of this material is abnormal. Thus the factors which normally cooperate to maintain constant the sugar concentration of the blood—however great may be the ingestion of carbohydrate—are defective in diabetes. The sugar which normally should be stored until required for combustion is poured into the circulation, giving rise to hyperglucemia and glucosuria.

As previously stated, the normal concentration of sugar in the blood varies from .07 to .11 per cent. In diabetes sugar concentrations of .15 to .25 per cent. are not uncommon and values as high as 1.0 per cent. have been recorded. Since hyperglucemia is the immediate cause of glucosuria, one might expect these two conditions to be parallel in their variations. This, however, is not always the case. In the early stages of diabetes, a slight hyperglucemia may be associated with marked glucosuria, an indication of active elimination of sugar by the kidneys. In the later stages the kidney may eliminate sugar less readily, and hence a strong hyperglucemia may be accompanied by but a relatively mild glucosuria. It is apparent that frequently a blood analysis excells an examination of the urine as a criterion upon which to base our judgment as to the severity of the disease. It may be noted that in diabetes complicated with nephritis one frequently observes a relatively small elimination of sugar in the urine.

The perfectly healthy active individual can burn as much as a kilo of glucose during the day. Some mild cases of diabetes can burn 100-150 grams of glucose derived from ingested carbohydrate plus the sugar formed from protein of the diet (about 50 grams). It may be pointed out that certain varieties of starch are more thoroughly utilized than others. Thus the diabetic tolerates oat meal starch very much more readily than any other. As the case becomes more severe, ingested sugar cannot be burned and is entirely eliminated, but the sugar of protein origin is still available for combustion. In a later stage even the sugar from this source cannot be burned. How-

ever, if the protein intake be low and the fat intake high, many of these cases can be rendered aglucosuric, combustion being in great part supported by the fat. In other instances, under no circumstances can glucosuria be avoided. Moreover, as the disease becomes more severe, less reliance can be placed upon fat combustion as this is defective. Hence greater protein intakes are called for.

It is probably true that in the last stages of diabetes there is no combustion of glucose or storage of glycogen or fat, yet all of these functions may be retained in part for a considerable period of time. Occasionally the ability to form fat is retained even in severe diabetes, resulting in obesity; but later this function also is lost, and one observes the characteristic condition of emaciation. The curve of development of a case of diabetes does not always follow a perfectly regular course. There are days during which a relatively good storage of glycogen is affected, only to be followed by less fortunate days. This occasional recovery of glycogenesis is to be welcomed since it spares the burning of fat. When the glycogen reserves are reduced to a very low ebb, combustion of fat becomes a necessity, and since this is frequently defective, there is the imminent danger of a severe or even fatal acidosis.[1]

PROTEIN METABOLISM IN DIABETES.

The total heat production of the diabetic is not below that found in a normal individual—in fact it is slightly greater. This is of interest in view of the important source of heat lost in the sugar eliminated in the urine. The loss is made good by the combustion of protein and fat; and the smaller the proportion of utilizable sugar obtained from the protein, the greater must be the intake of the latter in order that nitrogenous equilibrium may be maintained. On account of loss of glucose derived from protein, the calorific value of the latter may be reduced 50 per cent. The presence of a suitable quantity of protein in the diet is desired, since in the absence of sufficient of this exogenous material, the body will draw upon its own tissue protein in order to maintain proper heat production. A normal individual can maintain nitrogen equilibrium on as little as 7 grams or less of

1. A more detailed consideration of acidosis will be presented in the following chapter.

nitrogen daily, provided that sufficient carbohydrate and fat are also ingested, these latter materials acting as protein sparers. In a severe case of diabetes, however, where the ability to burn sugar is greatly reduced and likewise fat combustion is abnormal, a greatly increased ingestion of nitrogen will be required. An intake of 20 or even 30 grams of nitrogen may be needed, and in very severe cases it is practically impossible to maintain nitrogenous equilibrium however great the nitrogen intake may be.

Reference has already been made to the derivation of sugar from protein. A brief consideration of this process follows. As described in an earlier chapter, protein during digestion is hydrolized into amino acids. After absorption the latter undergo a further cleavage into a nitrogenous fraction—ultimately eliminated in great part as urea; and a non-nitrogenous fraction, which is in part burned directly and in part converted into dextrose. We may be more specific. It is possible to state which amino acids are burned directly, and which are converted into dextrose. It has been shown that leucine, tyrosine and phenylalanine are oxidized directly through the diacetic acid stage (see p. 79); while glycine, alanine, valine, aspartic acid, glutaminic acid, histidine, proline and arganine can give rise to dextrose in the diabetic organism.[1] Normally this dextrose is burned or stored as glycogen or fat, but in severe diabetes it is almost completely eliminated in the urine. Calculations reveal the fact that, were all the carbon of the protein converted into glucose, one gram of protein nitrogen would be equivalent to eight grams of glucose, the "glucose: nitrogen ratio" being 8:1. As a matter of fact experimental studies indicate that not more than 4.5 grams of glucose are derived from the protein equivalent of one gram of nitrogen, yielding a G:N ratio of 4.5:1. The remainder of the carbonaceous derivatives of the protein is burned directly. In human diabetes Lusk has frequently found a G:N ratio of 3.65:1, which he has regarded as the "fatal ratio;" *i.e.*, when the case has progressed so far that as much as 3.65 grams of glucose, formed from the equivalent of one gram of nitrogen, are excreted, the prognosis is considered very bad. The estimation of this ratio for a diabetic on a protein-fat diet is obviously of great importance, since the finding of a smaller

1. Dakin: "Oxidations and Reductions in the Animal Body," 1912 p. 58; also *Jour. Biol. Chem.*, 1913, XIII, p. 513.

ratio is evidence that some of the sugar of protein origin is being burned. The progressive lowering of this ratio is to be taken as a favorable prognostic sign. Clinically, ratios as high as 8:1, 12:1 or even 14:1 have been reported. Since these ratios account for more glucose than could possibly have been derived from protein, the assumption has been made that fat was the source of this extra sugar. As a matter of fact these results cannot be accepted as evidence of the conversion of fat into sugar since adequate controls were frequently lacking. In order that the estimation of this ratio may be of value, it is necessary that the observation be carried over several days, the nitrogen intake accurately known, and that the diet consist of protein and fat only. Proteins of various origins differ in their ability to promote glucosuria. Thus meat is most potent in this respect, next in order being casein, egg albumin and vegetable proteins.[1] It is possible that the nature of amino acids of the different proteins may be the determining factor.

As above indicated, the ability to burn sugar is not always entirely lacking, and the estimation of the G:N ratio was suggested as a method for determining the extent to which this function was retained. Another means of learning this, and also of throwing light upon the character of metabolism in general, is the estimation of the "respiratory coefficient," that is $\frac{\text{expired carbon dioxide}}{\text{inspired oxygen}}$. For the combustion of sugar, this quotient is 1; for the combustion of fat, it is approximately 0.7 and in the case of protein the quotient is about 0.8. In diabetes, quotients have been obtained varying from 0.64 to 0.76. In general it may be said that as the case becomes more severe the quotient will become progressively lower although to this there are notable exceptions. For a more comprehensive discussion of this phrase of the subject, reference must be made to other works.[2]

For the mild or moderately severe case of diabetes, dietetic treatment offers some hope of at least a temporary improvement, but for the very severe case, the situation is indeed a delicate

1. von Noorden: New Aspects of Diabetes, "Post-Graduate Lectures," New York, 1912, p. 18.

2. Cf. A. E. Taylor: *loc. cit.*, p. 335; also Benedict and Joslin: Carnegie Inst. Washington, 1910, Pub. No. 136.

one. The abnormally high concentration of sugar in the circulation is detrimental to the tissues. Resistance is lowered and the patient not infrequently succumbs to tuberculosis or pneumonia. Wounds heal with difficulty and infections often terminate in gangrene. Neuritis, cataract of the eye, stomatitis, caries of the teeth, and furunculosis are also frequent afflictions of the diabetic. If the attempt is made to reduce the hyperglucemia by lowering the protein intake, there is the danger that the body will draw upon its own tissue protein to meet the fuel requirements, thus aggravating the condition of emaciation. Moreover, in such a case a greater fat ingestion would be called for and since the combustion of this material is incomplete, there is the possibility of the development of acidosis with the danger of an impending fatal coma. However, in spite of this, it has been found beneficial occasionally to introduce a day of starvation, as the resulting diminished hyperglucemia and glucosuria induce a temporary improvement in the tolerance for carbohydrate.

OTHER TYPES OF MELLITURIA.

Pentosuria.—Pentosuria, a rare condition, the cause of which is obscure, it is an anomaly of metabolism which tends to run in families, but is probably harmless. The pentose found in the urine is optically inactive arabinose. A small amount of pentose has been detected in the urine of some typical cases of diabetes. Pentosuria is to a large extent independent of the presence or absence of pentoses in the diet. This is true, at least, of the types of pentosuria just described which are probably idiopathic. However, certain cases of pentosuria have been reported which most likely owe their origin to pentoses of the diet.[1] Cherries and apples and other fruits have been known to give rise to pentosuria where the urine had been previously free from pentose.

Levulosuria.—Levulose—ingested in fruits or resulting from cleavage of cane sugar—is converted into glucose in the intestinal wall and liver. The ability of the liver to affect this transformation may be reduced, in which condition levulose may appear in the urine. In diabetes, the ingestion of levulose will exaggerate the glucosuria, but levulose itself may not be excreted

1. Cf. Neubauer-Huppert: "*Analyse des Harns*," Wiesenbaden, 1910, p. 356.

in even severe cases of diabetes, as apparently the liver retains for a long time the function of converting levulose to glucose. Evidence of levulosuria is therefore to be regarded as an unfavorable diagnostic sign, since it points to an additional hepatic defect. Levulosuria has been observed in a large number of instances of cirrhosis of the liver, although it is not confined to injury of this organ. The appearance of levulose in the urine following the ingestion of a definite quantity of this sugar (usually 100 grams) is regarded as a valuable test for chronic degenerative processes in the liver such as cirrhosis. Levulosuria has also been reported in cases of pregnancy and after administration of thyroid preparations; and a few instances of apparently idiopathic levulosuria have been recorded.

Galactosuria.—As far as we are aware, idiopathic galactosuria does not occur, but an alimentary galactosuria may be observed following the ingestion of a sufficiently large amount of galactose or lactose, from which galactose is formed during digestion. Even in healthy individuals, the administration of 40 to 100 grams of galactose may be followed by galactosuria. In cirrhosis of the liver the tolerance is said to be lowered, as little as 20 grams of galactose leading to galactosuria. It has been stated by some workers that the sugar frequently appearing in the urine of children suffering with intestinal disturbances is galactose together with lactose.[1] In diabetes, galactose intensifies the elimination of glucose in the urine.

Lactosuria.—Excepting glucosuria, lactosuria is perhaps the most common type of mellituria. Like cane sugar, lactose itself is not utilizable by the body. It must be subjected to digestive action whereby it is converted into glucose and galactose. Alimentary lactosuria may be easily produced by the ingestion of a large quantity of lactose on an empty stomach, although normally such a condition is not observed after partaking of even large volumes of milk. Gastro-intestinal disturbances in children are not infrequently accompanied by lactosuria. Lactose is formed in the mammary glands. If its elimination is impeded, it is forced back into the circulation and excreted in the urine, since lactose is not available to the organism. Lactosuria may ensue during the last months of pregnancy, but more frequently during the early period of lactation. The excretion of this

1. Cf. Neubauer-Huppert: *loc. cit.*, p.. 144.

sugar usually ceases after lactation has been established, but may appear for a time when nursing is suspended.

Saccharosuria.—It is only after conversion into glucose and levulose during digestion that cane sugar becomes available to the organism. Should cane sugar be ingested in such quantities that it is absorbed before sufficient time has elapsed for this transformation, it would appear in the urine. Usually as much as 200 or 300 grams of cane sugar may be taken without provoking more than a trace of saccharosuria.

Maltosuria.—Maltosuria, sometimes associated with glucosuria, occurs following the ingestion of excessive amounts of maltose. This sugar, unlike sucrose or lactose, can be converted to glucose by most of the tissues. Maltosuria has been observed in a few cases of diabetes.

LABORATORY PROCEDURES.

1. *Reduction Tests.*—The property possessed by glucose, in common with many other sugars, of taking up oxygen in alkaline solution, especially in alkaline copper solutions, has been generally employed as a means of detecting this substance in urine. Fehling's has been the most commonly employed solution since its introduction more than sixty years ago. This solution has recently been modified by Benedict[1] so as to greatly increase its delicacy and render permanent its keeping power. The strong alkali, potassium hydroxide, has been replaced by sodium carbonate which does not exert upon glucose the destructive action of the hydroxide. By replacing the Rochelle salt with sodium citrate the solution has been found to keep *permanently*. It is about ten times as sensitive to sugar in urine as Fehling's or Haines' solutions but unlike these latter solutions, not appreciably reduced by creatinine, uric acid, chloroform, or the simple aldehydes. When albumen is present in the urine, it is advisable to remove it by coagulation and filtration before applying either Fehling's or Benedict's test. The removal of albumen is essential in the case of Nylander's test, as this reagent gives a change of color with albumen similar to that with sugar.

1. Benedict: *Jour. Biol. Chem.*, 1909, V. p. 485; *Jour. Amer. Med. Assoc.*, 1911, LVII, p. 1193; Myers: *Münch. med. Wochenschr.*, 1912, LIX, p. 1494.

a. *Benedict's Test.*—About 5 cc. of the reagent[1] are placed in a test tube and 8 to 10 drops (not more) of the urine to be examined added, and the mixture boiled vigorously from one to two minutes. It is allowed to cool *spontaneously*. In the presence of dextrose, *the entire body of the solution* will be filled with a precipitate, which may be *red*, *yellow* or *green* in color, depending upon the amount of sugar present. If the amount of glucose be small (under 0.3 per cent.) the precipitate forms only on cooling. If no sugar be present, the solution either remains perfectly clear or shows a faint turbidity that is blue in color and consists of precipitated urates, which need, however, cause no confusion. Even very small quantities of dextrose in urine (0.1 per cent.) yield precipitates of surprising bulk with this reagent.

b. *Fehling's Test.*—Equal parts of the two solutions[2] are mixed together and preferably diluted with 2 to 3 parts of water. About 5 to 10 cc. of this solution are placed in a test tube, heated to boiling and 8 to 10 drops of urine added. If no reduction occurs, boil again. Allowances must, however, be made with Fehling's solution for reductions from uric acid, creatinine, etc., which are especially liable to occur with concentrated urines when the solution is boiled, subsequent to the addition of the urine.

c. *Nylander's Test.*—To 5 cc. of urine, add 10 drops of Nylander's reagent[3] and boil. In the presence of sugar, the solution turns yellow, and finally black, bismuth being precipitated.

1. Benedict's single qualitative solution is composed of 17.3 grams of copper sulphate, 173.0 grams of sodium citrate and 100 grams of anhydrous sodium carbonate (double the weight of the crystalline salt may be employed) made up to one liter with distilled water. In the preparation of the solution, the copper sulphate should be dissolved separately in about 100-150 cc. of distilled water and then added slowly with constant stirring to a filtered solution (about 800 cc.) of the other ingredients and finally made up to one liter.

2. Fehling's solution is composed of two solutions, equal parts of which are mixed as used. The cupric sulphate solution (a) contains 34.65 grams of cupric sulphate dissolved in water and made up to 500 cc., and the alkaline tartrate solution (b) 125 grams of potassium hydroxide and 173 grams of Rochelle salt dissolved in water and made up to 500 cc.

3. Nylander's reagent is prepared by digesting 2 grams of bismuth subnitrate and 4 grams of Rochelle salt in 100 cc. of a 10 per cent. solution of potassium hydroxide. The solution is then cooled and filtered.

d. *Barfoed's Test.*—This test serves to detect *monosaccarides.* Place about 5 cc. of Barfoed's[1] solution in a test tube and heat to boiling. Add the urine under examination slowly, a few drops at a time, heating after each addition. Reduction is indicated by the production of a red precipitate, which may not form until the tube has stood a few minutes.

2. *Phenylhydrazine Reaction.*—In a test tube prepare a mixture of 5 drops of phenylhydrazine (the base), 10 drops of glacial acetic acid, and 1 cc. (20 drops) of a saturated solution of sodium chloride, then add 5 cc. of urine and boil gently for a few minutes. In the presence of glucose, yellow phenylglucosazone crystals will appear on cooling, which may be readily identified under the microscope and by their melting point, (slightly above 200° C.). This test is a very delicate one and these characteristic crystals are specific of glucose (or levulose), hence this test is a very important one when it is desired to definitely identify the sugar.

3. *The Quantitative Estimation of Glucose in Urine.*—Three general procedures are commonly employed clinically for the estimation of glucose in urine, viz., titration, fermentation, and the polariscopic method. The titration method of Benedict[2], which is conceded to be far superior to the older titration methods of Fehling and Purdy, is perhaps the method of choice. This method as we have found[3] gives very excellent results and no special or expensive instrument is required. It is superior to the Lohnstein fermentation, because the results may be obtained at once (about 5 min. necessary). It is also superior to the polariscopic method in those instances when levorotatory substances (as β-hydroxybutyric acid) are present, thus necessitating a determination both before and after fermentation.

a. *Benedict's Volumetric Method.*—The procedure which has been found most convenient for the estimation of glucose in urine is as follows: The urine, preferably accurately diluted, if a large amount of glucose is indicated, is placed in an accurately graduated 25 cc. burette. Twenty-five cc. of the volumetric

1. Barfoed's solution is composed of 4.5 grams of neutral crystallized cupric acetate dissolved in 100 cc. of water to which 1.2 cc. of 5 per cent. acetic acid is added.

2. Benedict: *Jour. Biol. Chem.*, 1911, IX, p. 57; *Jour. Amer. Med. Assoc.*, 1911, LVII, p. 1193.

3. Myers: *Münch. med. Wochenschr.*, 1912, LIX, p. 1494.

solution[1] are pipetted into a 150 cc. Jena extraction flask as used in fat extractions, 5-10 grams of sodium carbonate and a bit of powdered pumice added. The mixture is heated to vigorous boiling on a wire gauze with small asbestos mat and the urine run in rapidly until a chalk white precipitate begins to form and then more slowly with continuous boiling, until one drop dissipates the last trace of blue color, indicating the end point. Chloroform must not be present in the urine and if present, must be removed by boiling. Benedict found that 25 cc. of the copper solution were reduced by exactly 50 mgms. of glucose or 52 mgms. of levulose, and we have ascertained that the value for galactose is 54 mgms. and for lactose 67 mgms.

b. *Polariscope Examination.*—To estimate the amount of glucose in urine polariscopically the urine must be perfectly clear. Usually this is not the case and to clear, shake the slightly acid urine with a pinch of basic lead acetate and then filter. Place the clear urine in the polariscope tube, make several readings and calculate percentage of glucose according to the following formula

$$\frac{\text{Observed Rotation} \times 100}{52.5^2 \times \text{length of tube}} = \text{percentage of glucose}$$

c. *Fermentation Method.*—The best fermentation instrument is the improved instrument of Lohnstein. This instrument reads to 10 per cent. of sugar and has two scales, one for room temperature and the other for the incubator. One-half cc. of urine is carefully placed upon the clean mercury and then the same amount of a yeast emulsion is added. The carefully waxed stopper is then inserted, the mercury column is set at 0, the weight placed on the stopper and the instrument allowed to incubate. Very accurate results are obtained with this instrument. The only disadvantages are the length of time required to obtain the result and the difficulty in cleaning the instrument.

1. Benedict's volumetric solution is likewise permanent and is composed of 18.0 grams of copper sulphate, 100 grams of anhydrous or double the quantity of crystallized sodium carbonate, 200 grams of sodium or potassium citrate, 125 grams of potassium sulphocyanate, and 5 cc. of 5 per cent. potassium ferrocyanide solution, made up to one liter with distilled water. In preparation the ingredients are dissolved in the same manner as the qualitative reagent, *i.e.*, the copper separately.

2. The specific rotation of d-glucose may be taken as +52.5°, of lactose as +53°, of d-galactose as +81° and of levulose as —92°, Cf. Tollens: *Abderhalden's Handbuch der Biochemichen Arbeitsmethoden*, 1910, II, p. 122.

Occasionally certain other substances occur in urine which may cause a confusion with glucose when the ordinary copper solutions are the only source of data. Among such are levulose, lactose, pentoses, galactose, conjugate glucuronates, homogentisic acid, etc. Fermentation, the polariscope and the phenylhydrazine reaction yield especially valuable data here. Glucose and levulose are both fermentable and both give the same ozazone. Levulose, however, is levorotatory and has specific reactions—Borchardt's, Seliawanoff's[1]. Lactose and pentose are not directly fermentable with yeast. Lactose does not give the Barfoed reaction, being a disaccharide. It may be partially identified by the mucic acid test[2], though galactose also gives this reaction. Its ozazone is not sufficiently insoluble to serve as a test in urine. The glucuronates and homogentisic acid are not fermentable with yeast. In the presence of glucuronates a levorotation will be observed after fermentation. Pentoses and the glucuronates both give Tollen's phloroglucin reaction,[3] but the pentoses only the orcin reaction,[4] which may be applied in the form of Bial's reagent. A urine containing homogentisic acid does not give the bismuth reaction, and furthermore, turns dark on standing.

1. When a few drops of urine are added to 5 cc. of Seliawanoff's reagent, (0.05 grams of resorcin in 100 cc. of dilute (1-2) hydrochloric acid) and the mixture boiled a red color and a red precipitate will be observed in the presence of levulose.

2. Upon evaporating 100 cc. of urine containing lactose with 20-25 cc. of concentrated nitric acid in a shallow, broad glass vessel over a water bath, until only about 20 cc. remain, fine gritty crystals of *mucic acid* should separate in the presence of lactose.

3. The phloroglucin reaction is performed by adding a little of the material to the urine mixed with an equal volume of hydrochloric acid (sp. gr. 1.09) and heating on the water bath. Pentose, galactose or glucuronic acid will be indicated by the appearance of a red color.

4. The orcin test may be performed in a similar manner to the phlorglucin reaction substituting orcin and heating the mixture to boiling in this case. Here the end color reaction is green. Bial uses as reagent 30 per cent. hydrochloric acid which contains one gram of orcin and 25 drops of a ferric chloride solution (62.9 per cent. of the crystalline salt) in 500 cc. of the acid. About 5 cc. of the reagent is heated to boiling and then a few drops (not more than 1 cc.) of the urine is added to the hot but not boiling liquid. In the presence of pentose the liquid turns a beautiful green.

CHAPTER VI.

ACIDOSIS.

In the course of the metabolism of the three foodstuffs, protein, carbohydrate and fat, acid substances are formed. Normally, however, it is only protein metabolism which contributes appreciable quantities of acid compounds for elimination in the urine, the acid substances incident to carbohydrate and fat metabolism representing for the most part intermediary changes, which eventually give rise to the end products, carbon dioxide and water. When, on the other hand, combustion of these materials becomes incomplete, the acid compounds of intermediary metabolism accumulate in the blood and are excreted in the urine. To this condition Naunyn has applied the term "acidosis."[1]

The acid substances or "acetone bodies" with which we are particularly concerned are acetone, diacetic acid and β-hydroxybutyric acid. Protein or more specifically certain amino acids[2] have been known to yield acetone bodies, but these substances are undoubtedly chiefly derived from incomplete fat combustion and hence a brief resumé of our conception of fat metabolism follows.

The changes taking place in ingested fat prior to its storage in the tissues have been described in Chapter II. As a preliminary to oxidation stored fat undergoes a cleavage into glycerol and fatty acid. The glycerol is converted to glucose which is metabolized in the usual way. The fatty acid fraction suffers repeated oxidation at the β-carbon atom until butyric acid is formed, from this stage on a somewhat different type of reaction taking place. From butyric acid is formed β-hydroxybutyric acid which is then further oxidized to diacetic acid, the latter then through the stages of acetic and formic acids finally giving

1. For a general and more extended discussion of acidosis, see A. E. Taylor: "Digestion and Metabolism" Philadelphia and New York, 1912. For an excellent review of the literature, reference may be made to James Ewing: *Arch. Int. Med.*, 1908, II, pp. 330, 448.

2. See page 70.

rise to the end products, carbon dioxide and water. Associated with this process there is a side reaction whereby diacetic acid is converted into acetone, although normally this occurs to but a slight extent, only the merest traces of acetone appearing in the urine.[1] However, when fat combustion suddenly becomes excessive, this side reaction is accentuated, the oxidation of diacetic acid and β-hydroxybutyric acid is incomplete, acetone bodies appear in the urine, and we have the condition, acidosis. An excessive combustion of fat becomes necessary for the proper maintenance of heat production whenever there is a paucity of carbohydrate, which is apparently more readily oxidized than fat. Thus starvation and the early period of a protein-fat diet are attended with acidosis, which quickly disappears after the administration of carbohydrate, and which in the case of the protein-fat diet may vanish spontaneously, the fat burning mechanism presumably having improved with use; or it may be that there is a better utilization of the carbohydrate of protein origin.

In the mild stages of diabetes where total withdrawal of carbohydrate is recommended, a marked elimination of acetone bodies is noted. However, as von Noorden[2] points out, this is transient and a physiological phenomenon, being observed even in normal individuals under similar conditions of diet. In the advanced stages of diabetes and in certain other conditions there is probably a specific defect in fat combustion, and the elimination of acetone bodies is but little influenced by the utilization of carbohydrate. If the individual suffering with severe diabetes could utilize fat in the normal manner, his condition might be greatly alleviated, but the defect in this process removes a very efficient source of heat, and gives rise to substances which contribute to the usually fatal outcome. The acidosis of diabetes diminishes whenever the carbohydrate tolerance improves, the change being probably due to the generally improved condition which is shared by the fat-burning mechanism. At any rate such a view is more acceptable than the rather vague expression of Rosenfeld that fat can burn only "in the flame of carbohydrates."

1 There is said to be a daily elimination of 0.01 gram of acetone from normal individuals.

2 For valuable suggestions on dietetic and alkali therapy in acidosis, reference may be made to von Noorden's "New Aspects of Diabetes," New York, 1912.

In severe diabetes it is probable that the oxidation of the fatty acid is normal down to the butyric acid stage. From here on the combustion may be incomplete or defective. The early period of the acidosis may be attended with merely a moderately strong acetonuria, although as the condition becomes more severe the precursors of acetone, viz.: diacetic and β-hydroxybutyric acids, likewise appear in the urine. In still later stages of the disease, oxidation of β-hydroxybutyric acid may be markedly diminished, in which case this substance appears in the urine in large amounts associated with only nominal quantities of diacetic acid and acetone. In acidosis, the elimination of 10 to 20 grams of acetone bodies is not uncommon, and figures as high as 50 or even 100 grams have been recorded.

The degree of acidosis in a diabetic on a protein-fat diet is influenced somewhat by the nature of the fatty acid fraction of the fat. For example, one molecule of stearic and palmitic acids each yields one molecule of butyric acid, while one molecule of oleic acid gives rise to more than one molecule of butyric acid. Thus fat mixtures fluid at room temperature—those containing a preponderance of olein, such as olive oil—yield larger quantities of acetone bodies than do the more solid fats, *e.g.*, the fats of beef and mutton. Butter which contains preformed butyric acid esters also tends to accentuate the condition of acidosis. Nevertheless it may be necessary to include butter and olive oil in the diet, as the continued use of beef or mutton fats is borne with difficulty.

It has been stated that the fatty acids are oxidized to carbon dioxide and water through the stages of butyric acid, diacetic acid, etc. This applies only to fatty acids with an even number of carbon atoms, such as the ordinary fatty acids of our dietary, stearic, palmitic and oleic acids. Fatty acids with an uneven number of carbon atoms cannot be transformed into butyric acid, but according to the recent studies of Ringer[1] they may be transformed into dextrose in so far as they are convertible into propionic acid. The oxidation of the higher fatty acids of this type to the propionic acid stage would thus furnish energy to the diabetic without contributing to the acidosis; on the contrary, there would be an antiketogenic influence.

The relation of the acetone bodies to coma is as yet not definitely understood, and it can hardly be discussed at length

1. Ringer: *Jour. Biol. Chem.*, 1913, XIV, p. 43.

in the present brief chapter. We can only point out that the toxic action is not due entirely to the acid nature of the acetone bodies, since their salts are also somewhat toxic. Furthermore, susceptibility to their toxic influence is evidently subject to great variations since large eliminations of acetone bodies are sometimes encountered with no indication of coma, and on the other hand, coma may occur with only a moderate acetonuria. The suggestion has been made that acidosis does not produce coma, but that both these conditions are results of some common toxic agent. It is not improbable that the influence of acetone upon respiration may play an important role.[1] The nephritis associated with long-continued acidosis may bear some relation to the development of coma.

The relatively large amounts of acid compounds in the blood and tissues induce the liberation of increased quantities of bases, principally ammonia; and indeed the estimation of ammonia often forms a simple and valuable guide in following the progress of a case of acidosis. Normally, 0.3 to 0.6 gram of ammonia is eliminated in the urine daily. In acidosis, outputs of 2 to 10 grams are not uncommon, and Magnus-Levy has calculated that every gram of ammonia in excess of the normal corresponds to approximately 6 grams of β-hydroxybutyric acid. It should be noted, however, that the ammonia excretion is not an infallible indicator of the degree of acidosis, since the quantitative output of ammonia is influenced by several other factors, *e.g.*, total nitrogen output, defective urea formation in the liver, etc. (See Chapter III.) At times, therefore, the direct estimation of the individual acetone bodies may be desirable. There is little difficulty in obtaining sufficient ammonia for neutralizing the abnormal acids, but it should be understood that in addition to ammonia, all cations of the body contribute to this neutralization, and there is thus the tendency to disturb the mineral equilibrium in the tissues. In all cases of marked acidosis, administrations of alkalies are indicated. The alkali—usually sodium carbonate or sodium bicarbonate—serves merely to facilitate the removal of the acid compounds but has no important influence upon their formation. The use of alkali has frequently given temporary relief, probably owing to the resulting diuresis

1. Cf. Henderson and Underhill: *Amer. Jour. Physiol.*, 1911, XXVIII, p. 275.

during which considerable toxic material was removed, but the alkali therapy has not given generally satisfactory results. Taylor[1] has made the interesting suggestion that the logical procedure would be to administer a mixture of the salts of sodium potassium and calcium rather than of sodium alone, as in this manner there would be more opportunity for maintaining mineral equilibrium in the tissues.

Acidosis may be a concomitant of a number of abnormal conditions in addition to diabetes. Its occurrence in starvation has already been noted. Here the eliminations of acetone bodies may be quite as high as in the severe acidosis of diabetes. Acidosis has been observed in eclampsia and pernicious vomiting of pregnancy, although in these cases, and especially in the latter it is probably to be attributed to the inadequate state of nutrition.[2]

Characteristic cyclic vomiting, occurring usually in children, is accompanied by acidosis. Recurrence is very common, each attack as a rule lasting two or three days, or occasionally as long as two weeks. The condition is apparently uninfluenced by the administration of carbohydrates. It has been argued by some that we have here a condition of acid intoxication, although Ewing is of the opinion that we are dealing with various disturbances of metabolism including defective hepatic function and poisoning with intestinal putrefactive products.

Acidosis following anesthesia is also well recognized, chloroform being more potent in this respect than ether. In some cases only a mild acetonuria is observed, while in others, especially in children, this urinary finding is associated with nausea, vomiting and somnolence, these symptoms lasting only a few days. In still other cases the condition may be serious indeed, involving jaundice, acidosis, convulsions and coma, usually terminating in death in a few days. In addition to the acetone bodies, ammonium lactate and increased amounts of amino acids appear in the urine. The condition is uninfluenced by the presence or absence of sugar in the diet, and is attributed to necrosis of the liver induced by the chloroform. Subjects predisposed to this condition are generally regarded as poor surgical risks.

In typhoid fever, scarlatina, diphtheria, measles and many other infections a marked acidosis has been observed, although

1. A. E. Taylor: *Loc. cit.*, p. 330.
2. Cf. Underhill and Rand: *Arch. Int. Med.*, 1910, V, p. 61.

the degree of acidosis bears no striking parallelism to the severity of the disease. Administrations of sugar sometimes do and at other times do not influence the course of the acidosis. These instances of acidosis are attributed in part to the element of starvation, and in part to the toxic destruction of tissues. A similar dual explanation is offered to account for the occurrence of acidosis following the use of many drugs, *e.g.*, phosphorus, arsenic and lead compounds, antipyrin, morphine, atropine, curare and carbon monoxide.

LABORATORY PROCEDURES.

In clinical work it will usually be found sufficient to employ qualitative tests for acetone and diacetic acid and when these tests indicate the existence of an acidosis, to estimate the degree of this acidosis indirectly with the simple formaldehyde titration method for ammonia.[1] For acetone, the Rothera test has been found very satisfactory, while the simple Gerhardt test for diacetic acid is sufficiently accurate in the probable absence of interfering drugs. Acetone, diacetic acid and β-hydroxybutyric acid may be directly estimated with great accuracy by the Folin, Folin-Hart and Shaffer methods, but time will hardly allow this in routine clinical work.

1. *Acetone.*—

a. *Rothera's Test.*[2]—To 5 to 10 cc. of urine, add about a gram of ammonium sulphate, 2 to 3 drops of a freshly prepared aqueous solution of sodium nitroprusside, and then 2 cc. of strong ammonium hydroxide, which may be stratified upon the urine. A positive reaction is indicated by the slow development of a characteristic permanganate color. The delicacy is 1 to 20,000.

b. *Gunning's Iodoform Test.*—To about 5 cc. of urine or distillate in a test tube, add a few drops of iodine-potassium iodine solution and enough ammonia to form a black precipitate of nitrogen iodide. Allow the tube to stand (the length of time depending upon the amount of acetone present) and note the formation of a yellowish sediment consisting of iodoform.

2. *Diacetic Acid.*—

a. *Gerhardt's Test.*—To about 5 cc. of urine, add ferric chloride solution, drop by drop, until no more precipitate forms. In

1. See Chapter III, p. 43.
2. Rothera: *Jour. Physiol.*, 1908, XXXVII, p. 491.

the presence of diacetic acid, a Bordeaux-red color is produced. This color may be somewhat masked by the precipitate of ferric phosphate, in which case the fluid should be filtered. A positive result indicates the *possible* presence of diacetic acid. A variety of drugs or their derivatives when present in the urine yield a similar reaction.

b. *Arnold-Lipliawsky Reaction.*—This reaction is somewhat more delicate than that of Gerhardt and much more specific. Five cc. of urine and an equal volume of the Arnold-Lipliawsky reagent[1] are mixed in a test tube, a few drops of ammonium hydroxide added, and the tube shaken vigorously. A brick red color will be produced. Ten to 20 cc. of hydrochloric acid (sp. gr. 1.19) are added to 1 to 2 cc. of this colored solution, then 3 cc. of chloroform, 2 to 4 drops ferric chloride and the fluid carefully mixed without shaking. Diacetic acid is indicated by the chloroform assuming a violet or blue color. If diacetic acid is absent, the color may be yellow or light red.

3. *β-hydroxybutyric Acid.*—To 20 cc. of urine, in an evaporating dish add 2 drops of acetic acid, and concentrate at a *gentle heat* until the volume is reduced to about 5 cc. Acidify the residue with a few drops of concentrated hydrochloric acid, add plaster of Paris to make a thick paste, and allow to partially "set." Then break up with a stirring rod and extract twice with ether by stirring and decantation. Pour off ether and evaporate spontaneously or over the water bath. Dissolve the residue in water, filter, and divide between two test tubes. To one tube, add 1 cc. hydrogen peroxide, and warm gently for about one minute, and then allow to cool. Add to each test tube about one gram of ammonium sulphate, a few drops of a freshly prepared water solution of sodium nitroprusside and overlay the solutions with 2 cc. of concentrated ammonium hydroxide as in the Rothera test for acetone. Allow the tubes to stand for four hours. At the end of this time compare. The tube to which the peroxide was added will show a purplish red contact if β-hydroxybutyric acid was originally present. If both tubes

1. The Arnold-Lipliawsky reagent consists of two separate solutions "a" and "b" which are mixed in the ratio of 1 : 2 just prior to use. "a" is one per cent. solution of potassium nitrite and "b" is one per cent. aqueous solution of *p*-amino-acetophenon, to which just enough hydrochloric acid has been added (about 2 cc.) drop by drop, to cause the solution, which is at first yellow, to become entirely colorless.

are now shaken, the difference in color will be seen throughout the fluid. Albumin, if present, should be removed.

4. *Determination of Acetone and Diacetic Acid. Folin-Hart Method.*—The same type of apparatus is here employed as used in the estimation of ammonia by the Folin method,[1] except that in the place of the aerometer cylinder, a large test tube about two inches in diameter is employed. The method: An excess of N/10 iodine solution and an excess of 40 per cent. potassium hydroxide are accurately measured into a wide-mouthed bottle containing 200 cc. of water. An aerometer cylinder containing alkaline hypoiodite solution is arranged to absorb any acetone which may be present in the air of the laboratory, and between the cylinder and the bottle the large test tube is suspended. This test tube should contain 20 cc. of the urine to be examined, 10 drops of 10 per cent. phosphoric acid, 10 grams of sodium chloride, and a little petroleum. It should be raised sufficiently high to facilitate the easy application of heat. Otherwise, the arrangement is the same as for ammonia, except that the air current is passed more slowly (and only for 25 minutes) to remove *acetone*. At this point the absorption bottle is removed and another substituted. The contents of the large test tube are now heated just to the boiling point and after a five minute interval again heated. At the end of the second twenty-five minutes, the *diacetic acid* will have been transformed to acetone, removed to the absorption bottle and there held as iodoform.

Titrate the excess of iodine in both absorption bottles until a light yellow color is observed. At this point a few centimeters of starch paste should be added, and the mixture titrated until no blue color is visible.

One cc. of N/10 iodine is equivalent to 0.967 milligram of acetone. The diacetic acid may be conveniently recorded in terms of acetone.

5. *Determination of β-hydroxybutyric Acid.*—

For the most accurate purposes, the method of Shaffer, in which the β-hydroxybutyric acid is oxidized to acetone and ultimately titrated as above, is perhaps the method of choice. For practical purposes, however, the method of Black is more easily carried out and is likewise accurate. The method: Fifty

1. See Chapter III, p. 44.

cc. of urine are rendered faintly alkaline with sodium carbonate, evaporated to one-third the original volume, and finally concentrated to about 10 cc. over a water bath. The residue is cooled, made distinctly acid with hydrochloric acid and enough plaster of Paris added to form a thick paste. When the mass has begun to " set," reduce it to the consistency of coarse meal with a heavy glass rod, and extract in a Soxhlet apparatus with ether for two hours. Now evaporate the ether in the air, dissolve the residue in water, filter (adding a little bone-black if necessary to obtain a clear filtrate) and make up to a known volume with water (25 cc. or less). Ascertain the amount of β-hydroxybutyric acid in the 50 cc. of urine by its rotation. The specific rotation of β-hydroxybutyric acid is $-24.12°$ for solutions of 1 to 11 per cent.

CHAPTER VII.

PIGMENTURIA.

A variety of pigments and pigment-forming substances appear in the urine under both normal and pathological conditions. Those which merit consideration are urochrome, urobilin, uroerythrin, bile pigments, hematoporphyrin, hemoglobin, methemoglobin, and melanin, all of which, except the last, having a common origin, viz., the blood pigment. Certain substances as homogentisic acid, hydrochinon and catechol, when present render the urine dark upon standing, while under appropriate conditions certain other substances develop pigments, or yield color reactions. Among such, may be mentioned the formation of indigo blue from indican under the action of oxidizing reagents, the formation of a red color by indoleacetic acid (urorosein) with hydrochloric acid in the presence of nitrites, Ehrlich's diazo reaction, and Ehrlich's aldehyde reaction, which is probably referable to the presence of urobilinogen.

Urochrome.—The yellow color of normal urine is probably dependent upon several pigments, but in greatest part upon urochrome. Urochrome is apparently closely related to urobilin, since the latter may be readily converted into urochrome through evaporation of its aqueous ether solution.

Urobilin.—When the excreted urine is exposed to the action of light, it is regularly found to contain a yellow pigment, urobilin, which is derived from a chromogen, urobilinogen, under the action of light and air. It is, however, claimed that no urobilin is present in freshly voided normal urine. Urobilin is probably similar to, if not identical with, the hydrobilirubin of the feces. Certain investigators have claimed that there are various forms of urobilin, *e.g.*, normal, febrile, etc. Urobilin is increased in most febrile diseases, erysipelas, malaria, pneumonia, scarlet fever, etc., also in cirrhosis of the liver, carcinoma of the liver, catarrhal icterus, pernicious anemia, appendicitis, etc. In general, disturbances of the liver and excessive destruction of red blood cells favor its increase. Poisoning with such drugs as acetanilid, which brings about a destruction of red cells,

may cause the elimination of enough urobilin to produce a urine almost black in color as has been found in a specimen examined by one of us[1].

Uroerythrin.—It is to this pigment that the beautiful red color often found in urinary sediments, especially urate sediments, is generally due. It frequently occurs, although in very small amounts, dissolved in normal urine. After great muscular activity, digestive disturbances, fevers, circulatory disturbances of the liver, and in many other pathological conditions, the elimination of uroerythrin is found to be increased.

Bile Pigments.—Bile pigments are not normally found in urine, and when present may be regarded as a symptom of disease. Of the bile pigments, bilirubin alone is encountered in freshly voided urine; other pigments, biliverdin, etc. being formed as oxidation products of the bilirubin on standing. Urine containing bile is yellowish-green to brown and upon shaking, the foam takes on a bright yellow color. Normally, bilirubin, which is formed from the blood pigment in the liver, is eliminated into the small intestine and there transformed to hydrobilirubin and excreted in the feces, while a certain portion after being reabsorbed into the blood is eliminated in the urine in the form of one of the normal urinary pigments. Whenever, for any cause, the outflow of bile is impeded, bilirubin is absorbed by the lymphatics and eliminated in the urine. This may be brought about by obstruction of the bile ducts, especially the common duct, due to simple swelling of its mucous membrane, as in catarrhal jaundice, to the presence of a biliary calculus, or to pressure by tumors in the duct or surrounding glands. Choluria also results in conditions where the blood pressure in the liver is lowered, *hepatogenic icterus*; also to an inability of the liver to transform the blood pigment to bile pigments as fast as it is brought to it, as in cases of acute yellow atrophy, pernicious anemia, etc., and is termed *hematogenic icterus.*

Hematoporphyrin.—Hematoporphyrin, a substance closely related to bilirubin, possibly an isomer, is occasionally found in the urine in various diseases, more commonly after the use of certain drugs, such as quinine, trional, and especially in sulfonal intoxication. In these conditions, the urine possesses a slightly reddish tint, although after sulfonal it may be more or less deep

1. See Gordinier: *Boston Med. Surg. Jour.*, 1911, CLXV, p. 202.

red. Here the color depends in greatest part, not upon the hematoporphyrin, but probably upon other reddish brown pigments.

Blood and Blood Pigments.—When urine contains blood from hemorrhage of the kidney, or other parts of the urinary tract, and the formed elements of the blood are found in the sediment, the condition is termed *hematuria*. In such conditions, when the quantity of blood is moderately large, the color of the urine may vary from a red to a dark brown. Where the hemorrhage is recent and the urine fresh, the color is a brighter red. In certain other conditions, however, the urine contains no red corpuscles, but only the blood pigment, hemoglobin, or as is often the case here, methemoglobin, which condition is termed *hemoglobinuria*. Two factors may play a part in bringing about hemoglobinuria, viz., hemolysis of the red blood cells and an inefficiency of the liver. The condition is observed after poisoning with chlorates and other drugs, after severe burns, and also in the periodic appearance of hemoglobinuria with fever.

Melanins.—In certain pathological conditions, viz., in the presence of melanotic tumors, dark pigments are sometimes eliminated with the urine. The freshly passed urine is generally clear, and upon standing, darkens and may become a very dark brown, or even black, indicating that the pigment is probably present in the form of a chromogen and is oxidized upon coming in contact with the air.

Indican.—By the action of putrefactive bacteria in intestines upon the amino acid, tryptophane, indole is set free. This indole is in large part absorbed by the blood, carried to the liver and there, after oxidation to indoxyl, combined with sulphuric acid and potassium to form indoxyl potassium sulphate or indican, which subsequently appears in the urine. Upon oxidation, indican yields indigo blue. It has conclusively been shown that indole is formed in the body by bacterial action only, although this action is not necessarily confined to the intestine, an increased indicanuria being observed in cases of empyema, putrid bronchitis, gangrene of the lung, etc. Normally, 5 to 20 milligrams of indican are eliminated in the course of 24 hours, but the amount may be enormously increased in conditions of excessive intestinal putrefaction. The hydrochloric acid of the gastric juice appears to regulate, to a certain extent, the degree

of this intestinal putrefaction, as in cases of anachlorhydria and hypochlorhydria, the amount of indican may be very greatly increased. We have found that in certain cases of pellagra[1] accompanied by anachlorhydria that the indican was enormously increased (250 mgms. per day in one case), while in those cases in which free hydrochloric acid was present, the indicanuria was much less marked. Another factor in this condition appeared to be the lack of pepsin. In cases where peristaltic movements have been impeded as in ileus and peritonitis, an increase in indican generally occurs, although this is hardly the case in simple uncomplicated constipation.

Indole-Acetic Acid (*Urorosein*).—Under certain bacterial conditions in the alimentary tract in various diseases, especially in cachectic conditions, a substance has been found to appear in the urine which becomes red when acidified with a mineral acid (hydrochloric) in the presence of an oxidizing agent (nitrites). This red pigment, previously called urorosein, has been shown by Herter to be indole-acetic acid.

Ehrlich's Diazo Reaction.—A chromogen sometimes appears in the urine under pathological conditions, especially in typhoid fever, which, when treated with diazobenzene-sulphonic acid and ammonia, produces a characteristic red color, visible in the foam on shaking. A positive reaction is obtained in the first two weeks of typhoid fever in about 75 per cent. of the cases. It usually disappears at the end of the third week of the disease, but generally reappears in a relapse. The intensity of the reaction, as a rule, runs parallel with the severity of the infection. The diagnostic import of the reaction in typhoid fever is lessened, however, by the fact that distinct reactions are frequently obtained in measles, scarlet fever, acute miliary tuberculosis, pneumonia, erysipelas, pyemia, etc.

Ehrlich's Aldehyde Reaction.—It has been shown by Ehrlich that under various pathological conditions, a deep cherry-red color will develop on shaking a specimen of urine with a few drops of an acid solution of *p*-dimethylaminobenzaldehyde, and that the resulting pigment can in part be extracted with chloroform. The reaction, according to O. Neubauer, is due to urobilinogen. Herter has also shown that the administration of skatole causes an intensification of the reaction. A positive reaction is commonly obtained in cases of tuberculosis, but more

1. Myers and Fine: *Amer. Jour. Med. Sci.*, 1913, CXLV, p. 705.

especially in conditions accompanied by a derangement of the liver cells, in which condition it is claimed by some clinicians to be of considerable diagnostic importance.

LABORATORY PROCEDURES.

In the examination of the various urinary pigments mentioned above, the spectroscope is of very great service. In fact, the identification of the specific absorption bands may be almost indispensable in certain cases. So far as possible, however, simple chemical tests have been outlined below.

1. *Urobilin.*—About 20 cc. of the urine under examination are acidified with a few drops of hydrochloric acid and shaken gently with 5 cc. of amyl alcohol. The amyl alcohol extracts the pigment, and when examined spectroscopically will show the characteristic urobilin absorption bands between *b* and *F* (the green and blue parts of the spectrum). Upon treating the amyl alcohol extract with an alcoholic solution of zinc chloride and ammonia, it will show a bright green fluorescence, and appear a faint pink color by transmitted light.

For a test for urobilinogen, see under Ehrlich's aldehyde reaction.

2. *Bile Pigments.*—Urine containing bile generally has a dark yellow color with a foam of bright yellow, which is valuable evidence as to the presence of bile.

a. *Gmelin's Test.*—This test consists simply in the careful stratification of urine upon concentrated nitric acid as in Heller's test for albumin, when, in the presence of bile pigments, various colored rings (green, blue, violet, red, and yellowish-red) will be noted at the point of contact.

b. *Smith's Test.*—If an alcoholic solution of iodine, having a good yellow color, is layered over urine containing bile, a green ring will be formed at the point of juncture in the presence of bilirubin.

3. *Hematoporphyrin.*—Hematoporphyrin is best identified spectroscopically. Caustic alkali of 10 per cent. concentration is added to an appropriate volume of urine in the proportion of 20 cc. to 100 cc. of urine. The hematoporphyrin, which is precipitated with the earthy phosphates, is filtered off, washed, transferred to a flask and warmed with a small amount of alcohol acidified with hydrochloric acid. Upon filtering, the solution will show the absorption bands of hematoporphyrin in acid solution.

4. *Blood Pigments.*—In conditions of *hematuria*, chemical tests for blood are usually superfluous, inasmuch as red blood cells can generally be detected in the urinary sediment upon microscopical examination. In *hemoglobinuria*, no erythrocytes are present, and it may be necessary to resort to a spectroscopic examination or certain chemical tests to ascertain the cause of the red to brown color which the specimen of urine under examination may possess. The spectroscope is particularly valuable here, because the absorption bands obtained from a direct examination of the filtered urine may enable one to decide at once whether the pigment is hemoglobin or methemoglobin, or to identify it as *e.g.*, hematoporphyrin without further examination. When it is simply necessary to identify the color as due to a blood pigment, the tests for " occult " blood may be applied in a similar manner to the directions given in Chapter II, p. 23. The guaiac test, for example, may be applied by simply adding 10 drops of the alcoholic solution of guaiac to 5 cc. of the urine and then 2 cc. of ozonized turpentine or hydrogen peroxide and allowing the tube to stand for two to three minutes. In the presence of blood, a blue color will develop.

5. *Melanin.*—In the presence of a melanin or melanogen, urine will show a black precipitate with ferric chloride or barium hydrate. Bromine water will also produce a precipitate which is yellow at first, but turns black on further standing. Care should be taken not to confuse melanin with an excess of indican.

6. *Indican.*—

a. *Qualitative Test.*—About 10 cc. of faintly acid urine are shaken with a small amount (0.1 gram) of basic lead acetate, filtered and the clear filtrate mixed with an equal volume of Obermayer's reagent[1] and about 5 cc. of chloroform. Upon shaking, the chloroform will assume a blue color if indican be present, the intensity of which will vary with the amount of indigo blue which has been brought into solution by the chloroform. Qualitatively the depth of color may be taken as indicating the degree of indicanuria. Normally, only a faint blue color is produced.

b. *Quantitative Estimation.*—The above qualitative test may be made the basis of a quantitative colorimetric method for the estimation of indican which we have found rapid and satisfac-

1. Obermayer's reagent is prepared by adding 2 to 4 grams of ferric chloride to 1 liter of concentrated hydrochloric acid.

tory. The quantity of urine to be employed depends upon the amount of indican present. Where qualitative tests have shown a strong reaction for indican, it will be found convenient to employ 35 to 45 cc. of urine. A little basic lead acetate is added to the faintly acid urine, the mixture shaken, filtered, and two 15 cc. or 20 cc. portions taken to serve as duplicates, and treated in separatory funnels with equal volumes of Obermayer's reagent, and an accurately measured amount of chloroform (10 cc.). The mixture is then shaken, and after the separation of chloroform, this is withdrawn, and fresh portions of measured amounts of chloroform added until the extracts become practically colorless. These extracts are then combined and filtered. The clear filtrate is compared in a Duboscq colorimeter with a standard solution of pure indigo blue[1] in chloroform. Results obtained by this method have been found to agree well with those obtained with the method of Ellinger. Only in a few instances out of

1. The use of a chloroform solution of indigotin as standard is to be preferred to the use of a standardized Fehling's solution. Fehling's solution was first employed as an empirical standard for comparison with indican by Folin (*Amer. Jour. Physiol.*, 1905, XIII, p. 53) and it was our original intention to ascertain the strength of this in terms of indigo blue and indican. A study of this question, however, showed us that proportionate changes in the depth of an indigotin solution did not show the same proportionate changes in matching up with Fehling's solution. Further, when Fehling's solution was diluted one-half with water and compared with a concentrated Fehling's, it did not show double the colorimetric reading. On this account, the use of Fehling's solution as a standard was abandoned. The standard indigotin was prepared by adding an excess of Kahlbaum's pure indigotin to warm chloroform, allowing it to stand over night, and then filtering to remove any suspended indigotin. The strength of this standard was then determined by titrating the indigotin solution with approximately N/400 potassium permanganate according to the method of Ellinger (*Zeitschr. f. physiol. Chem.*, 1903, XXXVIII, p. 178.) The method is as follows: 10 cc. portions of the chloroform extract are evaporated to dryness in small extraction flasks, treated with 10 cc. of concentrated sulphuric acid and heated on the water bath for five minutes. They are then treated with 100 cc. of distilled water and titrated with the permanganate until the last trace of blue color disappears and only a pale yellow remains. The permanganate is prepared by taking 5 cc. of a solution of pure potassium permanganate (3.0000 grams to 1 liter) and diluting to 200 cc. with water. One cc. of this solution is equivalent to 1.5 mgms. of indigotin. To obtain the value of the indigotin solution in terms of indican multiply by 1.92. This solution will keep for a considerable length of time in a well-stoppered bottle in the dark.

several hundred determinations has red pigment been encountered which interfered with the colorimetric estimation.

7. *Indole-Acetic Acid—Urorosein Reaction.*—To about 10 cc. of urine, add 2 cc. of concentrated hydrochloric acid and a few drops of a 1 per cent. solution of potassium nitrite. In the presence of indole-acetic acid a rose-red color will develop.

8. *Ehrlich's Diazo Reaction.*—Equal volumes of urine and the diazo reagent[1] are thoroughly mixed in a test tube by shaking and ammonium hydroxide quickly added in excess. The test is positive if the fluid assumes a deep cherry-red color, and the foam becomes a salmon pink. It should be borne in mind that the administration of certain drugs produces a similar reaction (napthalin) while others (tannic acid, gallic acid, etc.) diminish the reaction, or even cause it to disappear.

9. *Ehrlich's Aldehyde Reaction.*—About 5 cc. of urine are treated with 5 to 10 drops of the reagent,[2] agitated for a few minutes and the color noted. A positive reaction is shown by the development of a fine cherry-red color, due probably to urobilinogen. In case the test is applied for urobilinogen, it is important that the urine sample should be fresh and not long exposed to light.

1. The diazo reagent is composed of two definite solutions which are mixed just prior to use in the proportions of 1 part of (a) to 50 parts of (b). (a) is a 0.5 per cent. solution of sodium nitrite and (b) is a 0.5 per cent. solution of sulphanilic acid in 5 per cent. hydrochloric acid. The *p*-aminoacetophenon solution employed for the Arnold-Lipliawsky diacetic acid test may be substituted for the sulphanilic acid solution.

2. The reagent is a solution of *p*-dimethylaminobenzaldehyde in a mineral acid. A 2 per cent. solution in equal parts of water and concentrated hydrochloric acid is convenient for this purpose.

CHAPTER VIII.

Examination of Urinary Sediments.

The diagnostic value of the microscopic examination of urinary sediments is well recognized. In many instances it serves not only to disclose some inflammatory lesion in the urinary tract, but, taken together with the clinical symptoms, enables one to determine the seat of the lesion, and to follow the progress of the case.

The two general types of sediment constituents are the organized (formed elements) and unorganized (crystalline and amorphous material), the more important of which are included in the following lists.

Organized Constituents.	*Unorganized Constituents.*
Casts.	Ammonium magnesium phosphate (" triple phosphate").
Cylindroids.	Calcium oxalate.
Leucocytes (pus cells).	Calcium phosphate.
Erythrocytes.	Calcium carbonate.
Epithelial cells.	Uric acid.
Mucous cylinders.	Sodium urate (amorphous and crystalline).
Spermatozoa.	Ammonium urate.
Bacteria, yeasts, animal parasites, etc.	Hippuric acid.
	Cystine, leucine, tyrosine.

ORGANIZED CONSTITUENTS.

Casts.—Casts are molds of the uriniferous tubules, resulting probably from the coagulation of an exudate within the tubules. They vary considerably in length and breadth, depending upon their place of origin in the tubules, but, for the most part, the width of an individual cast is uniform and the ends are rounded, although tapering and twisting forms are occasionally observed. *Hyaline casts* (Fig. 1) are made up of a practically uniform transparent pale matrix. At times granules appear at one end or are scattered in small numbers over the cast, giving

rise to *hyaline-granular casts*. The granules may completely cover the hyaline matrix, when the terms *finely granular casts* (Fig. 2) or *coarsely granular casts* (Fig. 3) are used, depending upon the size of the granules. If morphological elements or fat droplets are found adhering to the casts in sufficient numbers, they are given corresponding names, as for example, *leucocytic (or pus) casts, blood casts, epithelial casts* and *fatty casts*. *Waxy casts* (Fig. 2) are light yellow and are somewhat larger and have more clearly defined outlines than do hyaline casts. *Brown granular casts* are relatively short and broad and appear to have broken ends. They probably result from disintegration of the epithelial lining of the tubules.

Cylindroids, False Casts, Mucous Cylinders.—Cylindroids (Fig. 6) resemble casts in structure, but are much longer and show tapering and branching. Cast-like formations consisting of amorphous urates may occasionally be mistaken for granular casts. Such masses, however, disappear on warming. Mucous cylinders are long tapering transparent bodies, which are, as a rule, much thinner than casts or cylindroids.

The appearance of casts in the urine cannot always be interpreted as evidence of a distinct nephritis. They may occur in the urine of apparently healthy individuals after cold baths or severe muscular exercise, just as albuminuria may follow these activities; and they may be present in small numbers in the urine of many presumably normal people past middle life who do not lead especially active lives. Nevertheless it would seem reasonable to suppose that cylindruria indicates some renal abnormality, which, however, may never be attended by definite clinical symptoms. It may be that the pathological condition is confined to a few small areas in the kidney. On the other hand, post mortem examinations have frequently revealed pathological alterations in the kidney, although the urine had been free from casts. In chronic interstitial nephritis, casts may be absent from the urine for long periods of time, while parenchymatous nephritis is quite regularly accompanied by cylindruria. Renal disturbances resulting from infectious diseases cause numerous casts to appear in the urine. The urine of the first day or two after ether anesthesia contains large numbers of casts, which, however, rapidly disappear. With regard to the particular type of cast observed in various conditions, it may be mentioned that the hyaline and granular varieties occur in almost any renal

disturbance; blood casts are looked upon as characteristic of acute diffuse nephritis and acute congestion of the kidney; fatty casts are associated with fatty degeneration of the kidney; and waxy casts are characteristic of amyloid disease. Cylindroids appear in essentially the same conditions as do hyaline casts.

Leucocytes.—Normal urine contains only a very small number of leucocytes (Fig. 4), and hence any noteworthy increase may be looked upon as evidence of an inflammatory process somewhere in the urinary tract, excepting in females where the presence of leucocytes may be due to contamination with vaginal discharge. For purposes of diagnosis, it is desirable to learn the origin of the leucocytes. When of renal origin, they are often associated with renal epithelial cells and casts. Catheterization of the ureters or bladder will frequently aid in determining whether the pyuria is due to pyelitis, ureteritis, cystitis, or urethritis. Chronic nephritis is not ordinarily attended with marked pyuria though large numbers of leucocytes may be observed in the acute condition, in an acute exacerbation of a chronic case, or where there exists a complicating inflammatory process in some other part of the urinary tract. Pus appears in renal tuberculosis in variable amounts—from only a few leucocytes to a distinct sediment of pus. Where a pyelitis is unilateral, the affected side may be occluded, and so for a time clear urine will be obtained. In an advanced case of cystitis, where the urine has become alkaline, the leucocytes may be decomposed into a mucous-like mass.

Erythrocytes.—Erythrocytes (Fig. 5) practically never appear in normal urine, and, therefore, when present, some pathological condition may be presumed to exist. When only a small number of red cells are present, the urine does not present an unusual appearance, and their detection may require the microscope. When blood is present in considerable amount, the color of the urine may vary from bright red to dark brown, depending upon the length of time the blood has been in the urine. Here again it is important to learn the source of the hematuria. When of renal origin, the red cells are often associated with casts and renal epithelial cells; the blood is usually intimately mixed with the urine and contains "blood shadows," corpuscles from which the hemoglobin has been washed out. Absence of these associations will often serve to eliminate the kidneys as the source of the bleeding. Renal hematuria may be due to simple hyperemia, renal tuberculosis, nephritis, infectious diseases, and numerous other conditions.

FIG. 1.—Hyaline Casts.

FIG. 2.—Finely Granular Casts also Pus Cast and Waxy Cast

FIG. 3.—Coarsely Granular Casts.

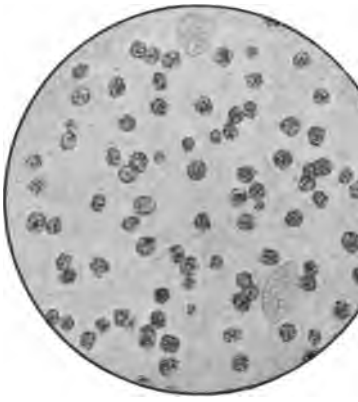


FIG. 4.—Pus Cells with Epithel Cells and Stringy Mucus.

FIG. 5.—Erythrocytes and Leucocytes.

FIG. 6.—Various Types of Epith Cells; also Cylindroid.

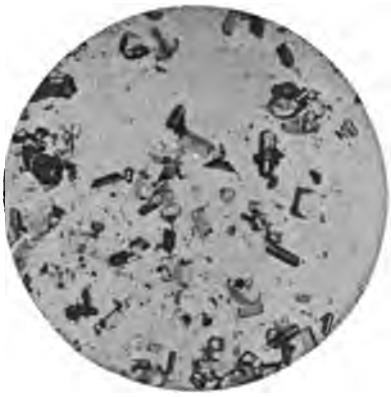


FIG. 7.—Ammonium Magnesium Phosphate Crystals with Amorphous Deposit.

FIG. 8.—Calcium Oxalate Crystals.

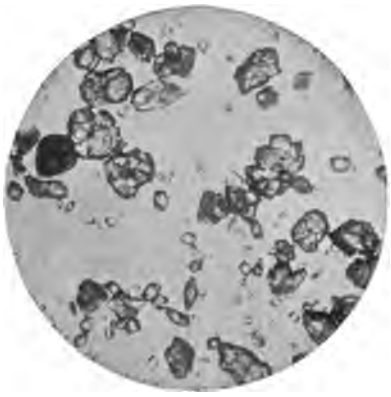


FIG 9.—Uric Acid Crystals.

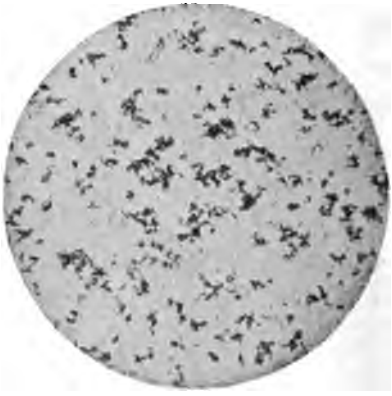


FIG. 10.—Amorphous Urates.

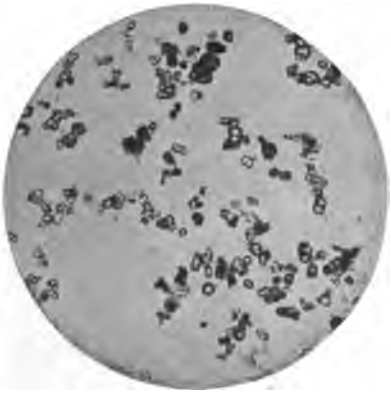


FIG. 11.—Ammonium Urate Crystals

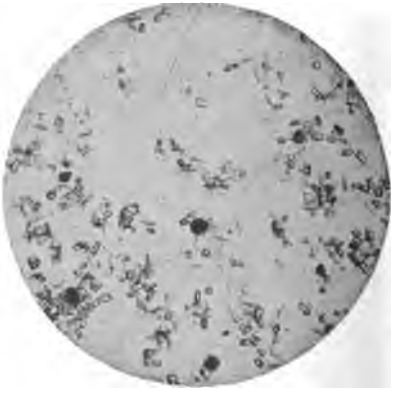


FIG. 12.—Calcium Phosphate and "Triple Phosphate" Crystals.

Blood cells from the urethra, prostate, or bladder usually present a normal or crenated appearance.

Epithelial Cells.—Under ordinary conditions the urine contains a small number of epithelial cells (Fig. 6), and when there is any notable increase, some abnormal condition is indicated. It should be remembered that in the female considerable numbers of pavement epithelial cells from the vagina are present in the urine, if voided. Three types of epithelial cells may be observed in urine, *viz*: round cells, caudate cells and large flat cells. Round cells are slightly larger than pus cells and contain large, clearly defined nuclei. They are found in the uriniferous tubules and in the deeper layers of the mucous membrane of other parts of the urinary tract. Caudate cells are derived from the superficial layers of the kidney pelvis and the neck of the bladder. Flat cells may be derived from the ureters, bladder or vagina. Attempts have been made to locate the abnormal condition by the type of cell appearing in the urine, but it cannot be said that any one type of cell is characteristic of a definite region. However, certain associations often aid in diagnosis. Thus, when round cells predominate and are accompanied by casts and albuminuria, the cells are probably of renal origin. When casts and albuminuria are absent, the simultaneous presence of large numbers of pus cells points to the kidney pelvis as the origin of the round cells.

In urine which has been exposed for some time after voiding or has been infected while in the bladder, a variety of bacteria may be observed. Yeasts cells showing characteristic budding are often noted in urine, especially in urine containing sugar.

UNORGANIZED CONSTITUENTS.

The identification of the various unorganized sediments is of much less importance diagnostically than that of the organized. However, they often aid in obtaining a picture of the condition, and in certain rare cases, where, for example, cystine, leucine or tyrosine appear in the urine, considerable importance may be attached to the examination.

The character of the sediment is for the most part determined by the reaction and concentration of the urine. Thus, uric acid, sodium urate, and calcium oxalate crystals are usually noted in acid urines; while crystals of triple phosphate, calcium phosphate, calcium carbonate and ammonium urate are observed in alkaline urines. Triple phosphate crystals are most abundant in alkaline

urines, although they are not infrequently encountered in urine of neutral or slightly acid reaction.

Simple inspection of the urinary deposit will often give a clue as to its nature. For example, a granular brick red sediment is referable to uric acid; a pink amorphous deposit, dissolving when warmed, indicates sodium urate; a white flocculent sediment, which disappears on the addition of acetic acid, points to the presence of phosphates or carbonates.

The crystallization of calcium oxalate, uric acid, etc., does not *necessarily* indicate an absolute increase in the elimination of these substances. It simply means that the conditions, such as reaction, concentration and temperature, were favorable for their crystallization. It is important to bear in mind that when such favorable conditions continually exist, there is the possibility of the formation of calculi.

Ammonium Magnesium Phosphate ("*Triple Phosphate*").—This compound (Fig. 7) crystallizes as prisms (" coffin lids ") and in a feathery arrangement, the former being the more characteristic. They are observed in urines which have been exposed for some time, and in disorders in which there is retention of urine in the bladder, as in cystitis and enlarged prostate.

Calcium Oxalate.—Calcium oxalate (Fig. 8) appears in the urine as octahedra of various sizes, and occasionally in a dumb-bell formation. The octahedral variety may at times be confused with small prismatic crystals of triple phosphate, and the dumb-bell type with calcium carbonate crystals. However, triple phosphate and calcium carbonate are soluble in acetic acid, while calcium oxalate is not. Calcium oxalate crystals may be noted after ingestion of tomatoes, rhubarb, garlic, asparagus, oranges., etc.; also in diabetes mellitus, phthisis, neurasthenia, etc.

Calcium Phosphate.—Calcium phosphate (Fig. 12) occurs in both amorphous and crystalline forms. The crystals are relatively long and wedge-shaped, often appearing in rosette arrangements. In this form they may resemble sodium urate crystals, but can be distinguished from the latter by their solubility in acetic acid.

Calcium Carbonate.—This substance may crystallize in the form of dumb-bells, which are somewhat smaller than the dumb-bell variety of calcium oxalte crystals.

Uric Acid.—Uric acid (Fig. 9) may crystallize in a number of forms, *e.g.*, wedge-shape, oval with pointed ends, irregular plates, and in various rosette arrangement of these forms. As a rule,

they are colored brownish-red, although perfectly colorless crystals may occasionally be observed. Uric acid crystals may appear in the urine after profuse perspiration, vomiting, diarrhea, and, in fact, in almost any condition giving rise to a concentrated urine.

Sodium Urate.—Sodium urate (Fig. 10) may occur either in an amorphous or a crystalline state. When crystalline, it appears in sheaves or clusters of colorless needles, resembling calcium phosphate crystals, from which they may be differentiated as already described.

Ammonium Urate.—This compound (Fig. 11) occurs in markedly alkaline urines as yellow spherical groups of very fine needles, or in a "thorn apple" form, which appears to be balls with spicules of various sizes attached.

Cystine crystallizes as thin colorless hexagonal plates; *tyrosine*, as long very thin needles grouped in sheaves; and *leucine*, as spherules resembling fat droplets, from which they can be distinguished by their insolubility in ether, and by the detection of concentric striations and radiating lines.

URINARY CALCULI.

In many instances, unorganized urinary sediments appear in the urine only after voiding. When such deposits regularly form within the urinary tract, they are liable to gather about some material which acts as a nucleus (mucus, epithelial cells, bacteria, etc.) and form calculi. Almost any of the unorganized sediment constituents may enter into the formation of such calculi, but calcium oxalate, calcium phosphate and uric acid are the most common. It has long been supposed that urinary calculi were largely composed of uric acid. Recent analyses, however, do not appear to entirely support this idea. Kahn and Rosenbloom[1] report analyses of sixteen renal calculi, which were composed mostly of calcium oxalate, and two cystic calculi which were almost pure uric acid. From the point of view of therapy, it is desirable to determine whether we are dealing, on the one hand, with uric acid, or, on the other, with calcium oxalate and calcium phosphate calculi, since diametrically opposite treatment should be instituted. The two types of calculi may be differentiated by their action on ignition and treatment with hydrochloric acid. Uric

1. Kahn and Rosenbloom: *Jour. Amer. Med. Assoc.*, 1912, LIX, p. 2252; also Rowlands: *Biochem. Jour.*, 1908, III, p. 346.

acid is insoluble in the acid and leaves but little residue on ignition; while the oxalate and phosphate of lime are soluble in this acid and are not appreciably affected by ignition. Since uric acid calculi are soluble in alkalies and insoluble in acids, whereas the reverse relation holds for the lime concretions, it is obvious that alkaline therapy cannot bring about solution of calculi made up for the most part of calcium oxalate and calcium phosphate, such treatment being indicated only for uric acid calculi. For consideration of other forms of calculi, and for details on the analysis of calculi, reference may be made to other works.[1]

LABORATORY PROCEDURES.

The urinary sediment should be examined as soon as possible after voiding. When a centrifuge is available this may be done at once, otherwise, it is advisable to allow the urine to remain for some hours in a conical sedimenting cylinder. Where this is done, it is necessary to take precautions to preserve the specimen, *e.g.*, by refrigeration, or with toluene, since casts and leucocytes are liable to undergo degenerative changes. The supernatant liquid in the centrifuge tube may be readily removed by simply inverting, most of the sediment remaining in the tip. The latter is then agitated, the one or two drops of sediment transferred to and spread out on a broad glass slide, and examined under the microscope. In searching for casts, the low power objective and high power eye-piece should be employed and the light reduced as far as possible with the iris diaphragm. This is especially important in the search for hyaline casts, as these appear as mere shadows. For a careful observation of erythrocytes, bacteria, etc., the high power objective is preferable; and if more than a mere inspection is to be made, it will be found convenient to employ a cover glass. When the casts are impregnated with red cells, leucocytes, epithelial cells, etc., they become more plainly visible. Occasionally deposits of phosphates may be mistaken macroscopically for pus, but the former will disappear on the addition of acetic acid, whereas the pus sediment is, if anything, accentuated under these conditions. Where similarity of crystalline forms causes confusion, advantage may be taken of solubility reactions as already described.

1. Purdy: Practical Uranalysis and Urinary Diagnosis, Philadelphia, 1901, p. 241; Wood: Chemical and Microscopical Diagnosis, New York, 1911, p. 608.

CHAPTER IX.

The Chemistry and Physiology of Milk.

From the chemical point of view, the differences observed in the milk of various species, are primarily quantitative, although these may assume considerable physiological importance. For this reason, attention will be devoted first to the properties and constituents of milk, irrespective of its origin, and then to a consideration of its adaptation to the nutrition of various animals.

Milk is essentially an emulsion of finely divided fat in a solution of protein, sugar, mineral matter, organic extractives and enzymes. It is the emulsified fat, together with a fine suspension of a casein-lime combination, which gives to milk its characteristic yellowish white non-transparent appearance. The specific gravity of milk varies between 1.028 and 1.034. The reaction of perfectly fresh milk is amphoteric toward litmus. Attempts have been made to determine the exact degree of acidity or alkalinity by titration methods, but it is probable that milk, like body fluids in general, is essentially neutral, that is, there is no noteworthy preponderance of the hydrogen ion or of the hydroxyl ion. On standing, there is a progressive increase in the acidity, owing to the action of bacteria upon the milk sugar by which lactic acid is formed. Fresh milk does not coagulate on boiling, but forms a thin pellicle consisting of casein and lime salts. As the bacterial formation of lactic acid progresses, the ease with which coagulation occurs increases, and finally, when the concentration of lactic acid is sufficiently great, coagulation takes place spontaneously at room temperature.

Qualitative Composition.—*Fat* exists in milk in the form of very small globules. Although the composition of milk fat (butter) varies considerably under different circumstances, it is composed chiefly of olein and palmitin, together with small amounts of the glycerides of butyric acid and of several other fatty acids. In addition to these there are present small quantities of lecithin, cholesterol and a yellow coloring matter. Attempts have been made to alter the proportion of olein, palmitin and stearin of ordinary beef fat in such a manner that when mixed with lard

and small quantities of milk and pure butter a product very similar to butter is obtained. This is known commercially as oleomargarine. The properties of the latter do in fact closely resemble those of butter, although it is very much lower in the glycerides of the volatile fatty acids, *e.g.*, butyrin. Nevertheless, disregarding the question of palatability, there appears to be no reason why such a preparation should not have a nutritive value equivalent to butter, and, indeed, for the diabetic the low concentration of butyric acid would make it preferable. (See Chapter VI.)

Protein is present in milk as casein and lactalbumin. The latter differs in no important detail from ordinary albumins, but the casein presents certain unique features. It belongs to the class of phosphoproteins, which are characterized by their content of phosphorus. Casein may be precipitated from milk by rendering the latter slightly acid with acetic acid, an excess of the acid subsequently dissolving the precipitate. Lactic acid resulting from bacterial action on lactose may likewise precipitate the casein. Casein thus prepared and dried is not appreciably soluble in pure water, but forms soluble combinations with alkalies and alkali earths, thus demonstrating its acid nature. Such a combination with calcium, mixed with calcium phosphate, presents a whitish opalescent appearance; and it is not improbable that the casein exists in milk in this form and so contributes to the opaque character of this fluid. The most characteristic property of casein is its coagulation under the influence of the enzyme, rennin, for which the presence of lime salts is essential. The enzymatic clotting of casein takes place in neutral medium and should be distinguished from acid coagulation. It is probable that the action of rennin and lime salts is distinct, the enzyme acting upon the casein to form the slightly hydrolyzed product " paracasein," which then forms an insoluble compound with the lime and is precipitated. This can be demonstrated by permitting rennin to act upon lime-free casein solutions. No precipitate is formed. If the mixture is boiled, thus killing the enzyme, and subsequently calcium salts added, the usual clotting is observed. A number of commercial preparations of milk proteins have appeared, *e.g.*, " nutrose "—sodium salt of casein; " eucasein "—the ammonium salt of casein; " plasmon "—a mixture of casein and sodium carbonate " lactalbumin " and " albuminlac."

The sugar of milk is lactose. Its molecule consists of one molecule each of glucose and galactose. Many of the properties of lactose have been described in Chapter V. Under the influence of certain bacteria, lactose is decomposed into lactic acid, to which milk owes its increasing acidity on standing. Owing to the action of certain yeasts and bacteria, a combination of alcoholic and lactic acid fermentation[1] of lactose is induced with the production of " kumyss " and " kephir," the former from mare's milk and the latter from cow's milk.

The mineral matter of milk consists of the phosphates and chlorides of calcium, magnesium, sodium, potassium and iron, although portions of some of these metals are probably in organic combination. Considerable interest is attached to the iron as it exists in the milk in extremely small amounts (0.0003–0.004 per cent.). This deficiency, however, is compensated by an abundant supply of iron in the infant's liver.

The organic extractives of milk include traces of urea, creatine, creatinine, orotic acid and citric acid.

Quantitative Relations.—For the quantitative composition of cow's milk, which has been the subject of more numerous analyses than any other, the following may be taken as average figures: water, 87 per cent.; protein, 3.5 per cent.; fat, 3.7 per cent.; sugar, 4.9 per cent., and ash, 0.7 per cent. It is interesting to note that the composition of the milk of various animals shows certain definite relations to the rate of growth of the young of the species. This is illustrated in the following table:

Species	Number of days required to double weight at birth	Percentage composition of milk (partial)		
		Protein	Ash	Sugar
Human	180	1.6	0.2	7.0
Horse	60	2.0	0.4	6.7
Cow	47	3.5	0.7	4.9
Goat	22	3.7	0.8	4.4
Sheep	15	4.9	0.8	4.0
Swine	14	5.2	0.8	4.0
Dog	9	7.4	1.3	3.2
Rabbit	6	14.4	2.5	—

1. Some protein decomposition also probably takes place.

It will be observed that for the infant, who doubles its initial weight in 180 days, the milk is relatively low in protein and mineral constituents, whereas for the rabbit, which grows very rapidly, doubling its weight in but six days, the protein and ash of the milk are very high. The animals intermediate in the list show corresponding variations. It is obvious that the more rapidly an animal grows, involving the formation of muscle and skeletal tissue, the greater will be its requirement of protein and mineral matter.

Of practical interest is the difference in composition between human and cow's milk. Although both types show marked variations in composition, it may be said that, in general, cow's milk contains twice as much protein, three times as much mineral matter, and considerably less sugar, than does woman's milk. Another important difference is the form of protein. Whereas cow's milk contains about 15 per cent. of its protein in the form of lactalbumin, the lactalbumin constitutes nearly 40 per cent. of the protein in human milk. Further, the fat of mother's milk differs from that of cow's milk in that it contains more olein. These differences become important considerations when it is desired to substitute cow's milk for mother's milk, certain modifications of the former being essential. Before further reference is made to methods of milk modification, attention will be called to a few of the fundamental metabolic requirements of the infant.

Heat production is proportional to the surface of the body rather than to body weight. Hence, since infants have greater surfaces relative to their body weights than do adults, it follows that they have a greater calorific requirement per unit weight. Thus a child under one year should receive at least 100 calories per kilo body weight, the calorific intake being gradually reduced until adult life, when 35 to 60 calories per kilo should prove adequate. It is important that the infant be supplied with a sufficient number of calories, as it is only under this condition that the maximum degree of nitrogen retention and the proper rate of growth can be attained. In human milk the protein supplies about 9 per cent. of the calories, and in general this relation may advantageously be preserved in the dietary throughout life. For example, for a man at moderately active work a diet containing 75 grams of protein and furnishing 3000 calories would

be ample. Here the protein supplies 10 per cent. of the total calories. Because of the relatively low concentration of sugar and relatively high concentration of protein in cow's milk, it is difficult to supply the infant with the requisite number of calories without at the same time giving too great a quantity of protein. Cow's milk contains about 20 per cent. of its calories in the form of protein, an amount larger than the infant can properly digest. Moreover, according to Howland,[1] when the proportion of protein reaches this figure, the specific dynamic action becomes very marked. Howland states that protein should normally furnish not less than 8 or more than 10 per cent. of the total energy of the food.

The " top milk " method is at present the most commonly employed procedure for modifying cow's milk, and is briefly as follows: the milk in a quart bottle is allowed to stand and accumulate its layer of cream in the upper part of the bottle. The composition of this layer is essentially altered only with respect to fat, which may reach 25 per cent., whereas the concentrations of protein, sugar, and salts remain practically unchanged. By utilizing the upper part of the contents of the bottle to varying depths below the layer of cream it is possible to obtain milk of any desired fat concentration. For the relation between depth and resulting fat concentration, we must refer to works on pediatrics.[2] By properly diluting milk of such known fat concentrations, one may obtain any desired concentration of protein and fat, the deficiency in sugar being made up by appropriate additions of this material. In many of the large cities there are laboratories, which undertake to supply milk of any composition according to the physician's prescription. However, the " top milk " method of modifying milk must still be generally employed, as such laboratories are not yet sufficiently widely distributed. In addition to the general laws of infant nutrition as above enunciated, there are numerous individual requirements of the infant which can be learned only by a careful study of each case. At times, for example, it may not be sufficient to simply make the percentage composition of cow's milk resemble mother's milk. Even when thus modified, the

1. Howland: *Amer. Jour. Dis. Child.*, 1911, II, p. 49.

2. Cf. H. D. Chapin and G. R. Pisek: " Diseases of Infants and Children," 1911, New York.

proportion of protein, or sugar, or fat may be too great or too small; or a sugar other than lactose (*e.g.*, cane sugar or maltose) may be better tolerated. There appears to be a tendency at present toward high protein feeding.[1] For further discussion on infant feeding we must refer to other works.[2]

Aside from the quantitative variations between human and cow's milk there are certain qualitative differences. The casein of woman's milk forms a much less dense coagulum under the influence of rennin than does cow's milk, a condition which accounts for the relative ease with which mother's milk is digested by the infant.

Secretion of Milk.—The initiation of milk secretion appears to be independent of the nervous system, although normally the latter undoubtedly exerts a regulatory influence. This view is the result of experiments on animals in which milk secretion was not prevented when all nerves to the mammary glands were cut. From the work of Starling and Lane-Claypon it would appear that the development of secreting cells is stimulated by some hormone influence. They found that injections of fetal extracts into virgin rabbits induce growth in the mammary gland, from which they concluded that normally the fetus elaborates a hormone which stimulates the growth of the mammary gland, but inhibits secretion. After birth, when the source of the hormone is removed, lactation starts, but is again arrested should pregnancy intervene before the end of lactation.

During the last few days of pregnancy and the first three or four days after parturition, the mammary glands secrete a fluid (colostrum), which differs from true milk. It is of a deeper yellow color than milk, and has a higher specific gravity which may be as great as 1.060. It is lower in fat and sugar than milk, but is considerably richer in protein, which accounts for its

1. At present pediatricians are not inclined to regard the high proportion of casein in cow's milk as detrimental to the digestive apparatus of the healthy infant, although prolonged high protein feeding would appear to maintain the intensity of metabolism at an unnecessarily high level. In the healthy infant there appears likewise to be no essential difference in the tolerance for the various sugars. (See Howland: Harvey lecture, 1913.)

2. Cf. H. D. Chapin and G. R. Pisek: *loc cit.*; also Langstein and Meyer: "Säuglingsernährung und Säuglingsstoffwechsel," Wiesbaden, 1910.

ability to coagulate on heating. In addition, colostrum contains an abundance of nucleated granular cells—the colostrum corpuscles. A few days after delivery the colostrum takes on the appearance and composition of true milk, although it may be several weeks before a constant composition is attained. During the first few weeks of lactation, the volume of the secretion is commonly as great as 400 cc., furnishing about 250 calories; but as the child grows, the secretion increases and may furnish as much as 750 calories.

Milk is the result of a specific secretory activity of the cellular elements of the mammary gland, filtration and diffusion being of secondary importance in this connection. This is indicated by the fact that milk contains lactose, whereas blood contains glucose. Moreover, casein is not found in blood, and lactalbumin is not identical with seralbumin. Again, according to Bunge, the mineral constituents of milk are present in different proportion than those in serum. The fat is no doubt likewise elaborated in the cells of the mammary gland, but may also be drawn from other fat depots in the body. As to the direct passage of food fat into the secretion there is considerable difference of opinion. Certainly some ingested substances can find their way into the milk. This is true of substances of the food which impart certain unusual flavors to the milk, and of drugs, *e.g.*, morphine, quinine, iodides, arsenic, lead, mercury, iron, etc.

With regard to the influence of food upon the composition of milk, we may say that an insufficient diet decreases both the quantity of milk and the solids, while both are increased by abundant food. Lactose appears to vary less under the influence of food than protein and fat. On the whole, the diet appears to be of secondary importance in this connection; the secretion of milk is an individual variable, and subject to nervous influences.

Sterilization and Pasteurization of Milk.—Milk properly sterilized in an autoclave is free from all living organisms, but simply bringing to a boil, as " sterilization " is ordinarily performed in the household, does not suffice for the destruction of spores, although the lactic acid producing organisms are probably destroyed. Thus milk after being subjected to the usual home sterilization is not so likely to " sour," but may, nevertheless, harbor numerous non-acid producing organisms. Heating

induces certain changes in the milk. The casein becomes less digestible, and undergoes partial decomposition associated with the liberation of a volatile sulphide. Any enzymes present would, of course, be destroyed. Exactly to what extent these changes are deleterious is not certain, but it is significant to note that a large proportion of cases of scurvy have been attributed to the use of sterilized milk. Pasteurization—heating to 68° C. for thirty minutes—kills most vegetative organisms but not spores. It has the advantage that there is less alteration in the milk constituents, but the keeping quality of such milk is not so great and it must therefore be preserved at a low temperature before consumption.

LABORATORY PROCEDURES.

The chemical examination of either human or cow's milk is necessarily a quantitative one, in which determinations of the various constituents, protein (casein and lactalbumin), fat, lactose and ash, are made to ascertain if they exist in the normal proportions. As has been previously pointed out, human milk normally contains 87 to 88 per cent. of water, 3 to 4 per cent. of fat, 5 to 8 (6 average) per cent. of lactose, 1 to 2 per cent. of protein and 0.2 to 0.4 per cent. ash. It has a pale blue color, a specific gravity between 1.028 and 1.032 and is amphoteric in reaction. The average composition of cow's milk from a large series of American analyses is 87.4 per cent. of water, 3.5 per cent. of fat, 4.5 per cent of lactose, 3.9 per cent. of proteins and 0.7 per cent. of ash, with a specific gravity of 1.031. Human milk is thus seen to contain more lactose and much less protein and inorganic salts. When an infant does not appear to be following the usual growth curve or where digestive disorders are manifest, an analysis of the milk of the mother may indicate the cause of the abnormality. One would thus be in a position to decide whether cow's milk should be substituted and to what extent the latter should be modified.

Holt describes a small milk-testing apparatus consisting of two cylinders, one for ascertaining the specific gravity, the other for roughly estimating the fat by the amount of cream which rises in a given length of time. This may in certain instances be of value from a clinical standpoint on account of its simplicity.

Even such simple determinations as the specific gravity often yield very valuable results, as in the detection of adulteration of cow's milk. It is likewise of importance at times to test for certain of the preservatives which are sometimes added to milk, such as formaldehyde, hydrogen peroxide and boric acid.

1. *Reaction.*—This may be determined qualitatively with red and blue litmus paper. Normally, it is slightly alkaline in its reaction to red litmus paper.

The amount of acidity may be titrated with N/10 NaOH, using phenolphthalein as an indicator. Ten cc. of milk are measured into an evaporating dish and diluted with 50 cc. of water and 2 to 3 drops of 1 per cent. solution of phenolphthalein added. The acidity may be expressed in degrees by considering each cc. of N/10 NaOH required to neutralize 100 cc. of milk as one degree. An acidity of 15 to 25 degrees may be observed within six hours after milking, and forty-eight hours after the acidity may be as high as 100 degrees. In buttermilk the acidity is generally between 100 and 125.

2. *Specific Gravity.*—The specific gravity of milk may be determined either with a special lactometer, or with an ordinary urinometer. The temperature of the milk should be 60 degrees F. A rough correction can be made by adding 0.0001 to the reading for each degree above 60 degrees F., and subtracting the same amount for each degree below.

3. *Total Solids.*—The total solids of the milk may be roughly calculated from the lactometer readings by the following formula suggested by Babcock. L = last two figures of the specific gravity corrected for temperature. F = the percentage of fat found in the milk.

$$\text{Total solids} = L/4 + 0.2\ F + F.$$

4. *Fat.*—The fat content in human milk may be determined with a fair degree of accuracy with the small Babcock tube which fits the conical cup of the ordinary medical centrifuge. It requires 5 cc. of milk, an equal volume of sulphuric acid, specific gravity 1.83, and enough of a mixture of equal parts of concentrated hydrochloric acid and amyl alcohol to fill the tube. Five cc. of the thoroughly mixed milk are pipetted into the tube, the above solutions added, the tube centrifuged for five minutes and the percentage of fat read off directly on the tube. Where

sufficient mother's milk is available, or in the case of cow's milk, the large Babcock tube for 17.6 cc. of milk should be employed as the results are more satisfactory. This amount of milk is placed in the tube with the special pipette, 17.5 cc. sulphuric acid, specific gravity 1.82 to 1.83, poured gently down the side of the tube and the tube mixed by a combination of a rotary and shaking motion. The tube is at once centrifugalized for five minutes, then boiling water added to the tube to bring the fat up into the graduated portion of the neck. The tube is again centrifugalized for one minute and the content of fat read off on the tube.

5. *Protein.*—

a. *Kjeldahl Method.*—The total protein may be accurately estimated by determining the nitrogen in carefully measured samples of milk (5 cc.) by the Kjeldahl method as previously described[1] under Urine. Human milk contains about 9 per cent. non-protein nitrogen and cow's milk about 6 per cent. This is deducted from the nitrogen obtained, and the result multiplied by 6.34 to ascertain the amount of protein.

b. *Precipitation with Phosphotungstic Acid.*—Five cc. of milk (or if necessary 1 cc.) are diluted with 9 parts of water and thoroughly mixed. This is poured into an ordinary Esbach albuminometer to the mark U and a phosphotungstic acid solution [2] added to R. The tube is then stoppered and inverted several times to insure a thorough mixing. It is now set aside for 24 hours at room temperature and the percentage of protein read off directly. This method gives a fair degree of accuracy, especially in woman's milk where the amount of casein is low.[3]

6. *Lactose.*—To 10 cc. of milk are added 10 cc. of the phosphotungstic acid solution employed above and 20 cc. of water. (If material available is insufficient, half quantities of the milk and other solutions may be taken.) After thoroughly mixing, this material is filtered through a dry filter. The filtrate comes through perfectly clear and the lactose content of this filtrate may be determined either polariscopically (specific rotation of lactose + 53.0) or by titration with Benedict's solution.[4] The

1. See Chapter III, p. 44.

2. The phosphotungstic acid solution is prepared by dissolving 70 grams of phosphotungstic acid in distilled water containing 20 cc. of concentrated hydrochloric acid and making up to one liter.

3. Cf. Boggs: *Johns Hopkins Hospital Bul.*, 1906, XVII, p. 342.

4. See Chapter V, p. 76.

titration is carried out the same as for glucose in urine. In this case, however, we have found that 0.067 gm. lactose is required to reduce the 25 cc. of Benedict's solution.[1] In making the calculation, the polariscopic figure is multiplied by four, or the titration figure divided by four, to allow for the dilution.

7. *Detection of Preservatives.*—

a. *Leach's Hydrochloric Acid Test for Formaldehyde.*—Add 10 cc. of the acid reagent[2] to an equal volume of the milk in a porcelain casserole, and heat slowly over the free flame nearly to boiling, holding the casserole by the handle, and giving it a rotary motion while heating to break up the curd. The presence of formaldehyde is indicated by a violet coloration, varying in depth with the amount present. In the absence of formaldehyde, the solution slowly turns brown. By this test 1 part of formaldehyde in 250,000 parts of milk is readily detected before the milk sours. After souring the delicacy is reduced to 1–50,000.

b. *Hydrogen Peroxide.*—Upon adding 2 to 3 drops of a 2 per cent. aqueous solution of *p*-phenylenediamine hydrochloride to about 10 cc. of milk, a blue color will immediately be produced in the presence of hydrogen peroxide upon shaking or allowing the mixture to stand for a few minutes. A delicacy of 1 part in 40,000 is claimed for this method.

c. *Boric Acid.*—The turmeric-paper test may be applied either to the ash or directly to the milk. In the latter case 10 cc. of milk are thoroughly mixed with 6 drops of concentrated hydrochloric acid, the turmeric paper moistened with the mixture and then dried. The presence of boric acid is indicated by the production of a deep red color, which is changed to green or blue upon treatment with dilute alkali. The test when properly applied has a delicacy of 1 part in 8000.

1. Myers: *Münch. med. Wochenschr.*, 1912, LIX, p. 1494.

2. Commercial hydrochloric acid (sp. gr. 1.2) containing 2 cc. of 10 per cent. ferric chloride per liter is used as reagent.

CHAPTER X.

Blood and Other Body Fluids.

The present chapter will be confined to a consideration of the *chemistry* of body fluids, although space will allow only a brief discussion of even this aspect of the subject. For this reason attention will be directed primarily to certain clinical possibilities opened up by several recent American investigations.

Blood.—From 6 to 8 per cent. of the weight of the body is made up by blood. In a certain sense it may be regarded as a fluid tissue, consisting of a transparent amber colored liquid (the blood plasma), in which are suspended an enormous number of formed elements—the erythrocytes, leucocytes and blood platelets. The specific gravity of blood varies between 1.045 and 1.075; for adult men the average is 1.058, and a little less for women. Blood is alkaline toward litmus, but from the physico-chemical point of view it is essentially neutral.

The chemical composition of blood plasma may be briefly outlined as follows:

- Plasma
  - Protein (fibrinogen)
  - Serum
    - Proteins: seralbumin, serglobulin.
    - Organic extractives: glucose, fats, lipoids, urea, uric acid, etc.
    - Salts: chlorides, carbonates, sulphates, and phosphates of ammonium, potassium, calcium, magnesium and iron.
    - Enzymes: thrombin, oxidase, catalase, amylase, lipase, etc., antienzymes.
    - Internal secretions, etc.

The most striking property of blood, or of blood plasma, is its ability to clot. This process involves the conversion of the

soluble protein, fibrinogen, into the insoluble protein, fibrin. Although there is still considerable difference of opinion, the mechanism of clotting is essentially as follows: The soluble fibrinogen is transformed into the insoluble fibrin through the agency of the enzyme, thrombin. The fibrinogen exists preformed in the plasma, whereas the thrombin is derived from a precursor substance—prothrombin; and for the conversion of prothrombin to thrombin the presence of calcium salts is essential.[1] As may be observed from the above outline, the fluid remaining after the precipitation of fibrinogen as fibrin is serum. The chemical composition of the blood serum is fairly constant, although in pathological conditions certain more or less definite variations from the normal may be encountered.

PARTIAL COMPOSITION OF BLOOD SERUM.[2]

	Per cent.
Total protein	7.0
Globulin	2.7
Albumin	4.3
Incoagulable and non-protein nitrogen	0.035
Ash	0.88
Chlorides	0.36

As may be calculated from the above, the globulin makes up about 38.5 per cent. and the albumin 61.5 per cent. of the total protein, yielding a ratio of globulin to albumin of 1 : 1.6. In the light of Epstein's recent studies this ratio with its pathological variations is of considerable interest. Epstein found that in certain diseases the proportion of globulin is much higher, although the total protein of the serum may be normal or even reduced. Thus he noted an increase in the globulin fraction in "(1) cardiac diseases associated with decompensation and serous effusions, (2) pulmonary or respiratory affections of inflammatory or non-inflammatory origin (pneumonia, emphysema, polycythemia), (3) diabetes mellitus, and (4) parenchymatous nephritis." In fact, in cases of parenchymatous nephritis the

1. For further discussion of the mechanism of clotting and the methods by which it may be hastened or delayed, see various papers by Howell and his co-workers, also Howell: Text-book of Physiology, New York and London, 1912.

2. Taken from Epstein: *Jour. Exper. Med.*, 1912, XVI, p. 720.

globulin was found to make up as much as 95 per cent. of the total protein. This observation may be of interest in connection with the large amounts of *albumin* generally present in the urine in this condition.

The globulin fraction was found normal or reduced in achylia gastrica, tuberculosis, diabetes insipidus, and chronic interstitial nephritis. Epstein further states that in those diseases associated with a relatively high content of globulin in the serum, there occurs an accumulation of water and salts. However, there are variations which cannot be accurately interpreted.

The concentration of the mineral constituents and non-protein nitrogen constituents (digestive and metabolic products) of the blood is assuming considerable importance at present as an index to the efficiency of the kidney as an excretory organ. Folin and Denis[1] reported that for a series of 16 normal individuals the non-protein nitrogen varied between 22 and 26 milligrams per 100 grams of blood, 11 to 13 milligrams per 100 grams of blood being in the form of urea. They also found uric acid present to the extent of 1 to 2 milligrams per 100 grams of blood. A marked increase in urea and total non-protein nitrogen was observed in syphilitics and insane patients, indicating some renal inefficiency. In cases of recognized chronic nephritis the total non-protein nitrogen rose as high as 96 milligrams per 100 grams of blood and the urea nitrogen 68 milligrams per 100 grams of blood. These cases are not necessarily associated with an increased concentration of uric acid. On the other hand, in many cases of gout the uric acid may accumulate in the blood to four or five times its original concentration and yet be accompanied by no marked retention of urea. In such cases the kidney is apparently inefficient only in so far as the elimination of uric acid is concerned.

As has been stated in Chapter V, in connection with a consideration of diabetes, blood normally contains approximately 0.1 per cent. dextrose, which may experience certain changes under special conditions as there outlined. Reference may be made to this chapter for cases in which a sugar determination may be desirable.

The color of the blood is due to the hemoglobin or oxyhemo-

1. Folin and Denis: *Jour. Biol. Chem.*, 1913, XIV, p. 29; see also Folin, Karsner and Denis: *Jour. Exper. Med.*, 1912, XVI, p. 789.

globin contained in the red blood corpuscles. This substance belongs to the group of combined proteins, and is a combination of a protein (globin) and an iron bearing coloring matter (hemochromogen), which is readily oxidized to hematin. Hemoglobin unites with oxygen in the lungs, forming oxyhemoglobin. This combination is a loose one, and while circulating in the capillaries through the tissues the oxygen is given up and reduced hemoglobin again formed. Hemoglobin is thus essentially an oxygen carrier and its ability to take up oxygen seems to be a function of the iron it contains. Venous blood owes its characteristic purple color to reduced hemoglobin, and after asphyxiation this substance is practically the only blood coloring matter present. Under certain conditions oxygen may become more firmly united to hemoglobin than is the case in oxyhemoglobin. The compound thus formed is methemoglobin, and can be detected in blood after poisoning with chlorates, nitrites, acetanilide and many other substances. Methemoglobin contains just as much oxygen as does oxyhemoglobin, but the oxygen is given up much less readily, and hence methemoglobin cannot replace oxyhemoglobin as an oxygen carrier. Other gases beside oxygen may combine with hemoglobin, *e.g.*, carbon monoxide, carbon dioxide, nitric oxide. Carbon-monoxide hemoglobin and nitric-oxide hemoglobin are very stable compounds, and of course cannot function as oxygen carriers.

Cerebrospinal Fluid.—There appears to be little difficulty in obtaining sufficient fluid for examination. Blatteis and Lederer[1] succeeded in removing fluid in all of their 426 cases, and Myers[2] states that 25 cc. of fluid were easily procured. After death, Myers was able to obtain large amounts of fluid—in one case over 200 cc. Normally, cerebrospinal fluid is clear and colorless, with a specific gravity of 1.005–1.008 and has a faintly alkaline reaction. Clear and colorless fluids are also observed in tuberculous meningitis, serous meningitis, hydrocephalus, tumors of the brain and various forms of mental disturbances. In the last mentioned cases, post mortem examinations usually reveal turbid fluids. Cloudy fluids are indicative of acute inflammatory processes.[3] The protein of cerebrospinal fluid is said to be, for

1. Blatteis and Lederer: *Jour. Amer. Med. Assoc.*, 1913, LX, p. 811.
2. Myers: *Jour. Biol. Chem.*, 1909, VI, p. 123.
3. Cf. Blatteis and Lederer: *loc. cit.*

the most part, a globulin, seralbumin being present only under exceptional conditions. Normally, the protein varies from 0.02 to 0.16 per cent., and is increased in syphilitic affections and tubercular meningitis, where it may reach as much as 0.3 per cent. Glucose is regularly observed in cerebrospinal fluid, and choline has been detected in several diseases, especially in dementia paralytica.[1]

Transudates and Exudates.—Normally, the serous membranes are moistened by fluids, which (except in the pericardial sac) are present in amounts insufficient for complete chemical analyses. In certain pathological states, considerable transudation may take place from the blood into the serous cavities, also into the subcutaneous tissues and under the epidermis. True transudates are, as a rule, poor in cellular elements and contain relatively little protein, while the transudates of inflammatory origin (the so-called exudates) are rich in leucocytes and yield more protein. The specific gravity of transudates varies between 1.005 and 1.015, and their protein content ranges from 1 to 2.5 per cent.; the specific gravity of exudates, on the other hand, may reach 1.030 and their protein concentration 4 to 6 per cent. In general, transudates and exudates are clear and present a light straw color. Occasionally an admixture of blood gives them a reddish tinge, in which case they are said to be hemorrhagic. Ascitic fluid may, through a rupture of a chylous vessel, become rich in finely emulsified fat, although ascitic fluid has been noted to have a chylous appearance without the presence of fat.[2]

LABORATORY PROCEDURES.

The laboratory methods which are described below have been included, not because of any simplicity, but because the data obtained with them may prove of positive diagnostic value, and have already yielded data of scientific interest. Numerous other determinations might be described, such as the estimation of hemoglobin, the specific gravity, lowering of the freezing point, etc. The estimation of hemoglobin belongs more properly to the subject of hematology, while the other tests may be

1. For further discussion on cerebrospinal fluid, see Myers: *loc. cit.*; also Simon: *Clinical Diagnosis*, Philadelphia and New York, 1911, p. 474.

2. For further discussion of transudates and exudates see Simon: *loc. cit.*, p. 456; also Hammarsten–Mandel: *Text-book of Physiological Chemistry.* 6th Ed. New York, 1911, p. 334.

found in any text-book on the subject, and have not been shown to be of any definite diagnostic value.

1. *Sugar*[1].—From 15 to 25 grams of fresh blood are transferred to a casserole containing about 150 cc. of phosphotungstic acid solution.[2] The weight of the blood sample is conveniently obtained by weighing the casserole with the phosphotungstic acid before and after the addition of the blood. The mixture is thoroughly stirred and boiled until the protein coagula coalesce into a single mass. The fluid is then decanted through a folded filter paper into a large casserole. Cold water is added to the mass of coagulum remaining in the original casserole, as this treatment renders the material brittle, and enables one to grind it with a pestel into small particles. In this condition the coagulum can be easily extracted with water, three successive extractions being usually sufficient to remove all the sugar. The clear filtrate with washings are treated with 10 per cent. NaOH until only a very faint acid reaction remains. This nearly neutralized solution is then evaporated on the water bath to about 50 cc., transferred to a 100 cc. volumetric flask and made up to the 100 cc. mark with water. The sugar is then determined by means of Allihn's gravimetric method, employing the two component solutions contained in Fehling's solution.[3] Place 30 cc. of the copper solution, 30 cc. of the alkaline tartrate solution and 60 cc. of water in a casserole of medium size, and heat to boiling. 25 cc. of the sugar solution are then added and the mixtures again brought to a boil and maintained at the boiling point for exactly two minutes. At the end of this interval the mixture is immediately filtered by means of suction through a weighed Gooch crucible containing asbestos, the red precipitate of cuprous oxide being transferred quantitatively to the crucible with the aid of hot water and a rubber tipped glass stirring rod. Alcohol is then added to facilitate drying and the crucible carefully heated in the upper part of the flame of a Bunsen burner. This ignition converts the red cuprous oxide into the black cupric oxide, ten minutes of heating usually being sufficient.

1. Bang has recently proposed methods for the estimation of sugar, also moisture, protein and chlorides, which require as little as 0.1 gram of blood. These microchemical methods described by Bang are apparently capable of yielding reliable data. See *Biochem. Zeitschr.*, 1913, XLIX, p. 19.

2. See foot-note Chapter IX, p. 112.

3. See foot-note Chapter V, p. 75.

The crucible is cooled and weighed. The difference between this weight and the original weight of the crucible represents the weight of cupric oxide, which should be multiplied by 0.9 to obtain its equivalent in terms of cuprous oxide. The amount of dextrose corresponding to this may be obtained from the tables published in the "Official and Provisional Methods of Analysis," pp. 50-51.[1] Multiplying this value by 4 gives the amount of sugar present in the sample of blood taken for analysis, from which the percentage concentration may be readily calculated.

Less accurately the sugar in the peripheral blood stream may be estimated by collecting 5 cc. of blood, and at once mixing with 100 cc. of absolute alcohol, filtering off the precipitate and washing with a little hot 95 per cent. alcohol. The filtrate is evaporated nearly to dryness in a small evaporating dish on the water bath, then completely transferred to a still smaller dish, washed with a little hot water, and evaporated practically to dryness. This is now completely washed with 5 one tenth cc. portions of boiling water into a Lohnstein saccharimeter, 0.5 cc. of yeast emulsion added, etc. as described in Chapter V. Since 5 cc. of blood have been employed the reading is divided by 10.

2. *Total Non-protein Nitrogen (Folin-Denis)*[2].—For the determination of the total non-protein nitrogen and also the urea in human blood, 5 cc. of blood are necessary. Folin and Denis suggest the following method for drawing the blood: An ordinary 5 cc. pipette is connected to a small sterile hypodermic needle with the aid of a short piece of pure gum tubing. The pipette should be perfectly dry and into the upper end is introduced a pinch of powdered potassium oxalate which is allowed to run down into the tip. The upper end is then connected with a rubber tube having a pinch cock. In this way exactly 5 cc. of blood may be drawn without clotting. The blood is then transferred at once to a 50 cc. volumetric flask half filled with methyl alcohol (acetone free). The flask is then filled up to the mark and vigorously shaken. Sometime after two hours the contents of the flask is filtered through a dry filter. To the filtrate is then added 2 to 3 drops of a saturated alcoholic solution of zinc chloride, and after standing for a few minutes, the mixture

1. This publication (Bulletin No. 107 revised) may be obtained from the U. S. Department of Agriculture, Bureau of Chemistry.

2. Folin and Denis: *Jour. Biol. Chem.*, 1912, XI, p. 527.

is again filtered through a dry paper. To determine the total non-protein nitrogen of the blood, 10 cc. of the alcoholic filtrate are transferred to a large Jena test tube (20–25 mm. by 200 mm.). One drop of sulphuric acid, one of kerosene and a pebble are added and the methyl alcohol driven off by immersing the test tube in a beaker of boiling water for five to ten minutes. When the alcohol is removed, 1 cc. of concentrated sulphuric acid, a gram of potassium sulphate,[1] and a drop of copper sulphate are added and boiled over a micro-burner for about two minutes after the solution becomes colorless. It is allowed to cool for about three minutes until it *just becomes viscous*, then diluted with about 6 cc. of water, adding the water slowly at first, then more rapidly to prevent solidification. To this mixture, after the necessary apparatus has been adjusted, is added an excess of sodium hydroxide (3 cc. of the saturated solution) and the ammonia removed either by aeration with compressed air or suction as described by Folin and Denis,[2] or by simple distillaton with a very small condenser into a second large test tube containing 1 cc. of N/10 acid and 2–3 cc. of water, as in the usual Kjeldahl distillation. About ten minutes usually suffice. The great delicacy of the method as described by Folin and Denis depends upon the determination of the ammonia thus obtained, with the aid of Nessler's solution, permitting the use of material containing considerably less than 1 mg. of nitrogen. For this determination in human blood 7 to 8 cc. of diluted Nessler's reagent[3] (diluted 1 to 5 just previous to use) are added and the

1. The necessity of having ammonia free reagents and distilled water is obvious.

2. For various detailed directions in this connection it may be necessary to consult their original paper.

3. Nessler's solution is prepared by dissolving 62.5 grams of potassium iodide in about 250 cc. of distilled water, setting aside a few cc. and adding gradually to the larger part a cold saturated solution of mercuric chloride (or mercuric iodide), of which about 500 cc. will be required, until the mercuric iodide precipitated ceases to be redissolved on stirring. When a permanent precipitate is obtained, restore the reserved potassium iodide so as to redissolve it, and continue adding mercuric chloride very gradually until a slight precipitate remains undissolved. Next dissolve 150 grams of pure potassium hydroxide in 150 cc. of distilled water, cool, add gradually to the above solution, and make up with distilled water to one liter. On standing, a brown precipitate is deposited, and the solution becomes clear, and of a pale greenish-yellow color. The clear supernatant fluid is decanted into a smaller bottle as required for use.

solution carefully washed into a 25 cc. volumetric flask and made up to volume. (If much ammonia is present, so that the resulting colored solution must be diluted to 50 or 100 cc., correspondingly large amounts of Nessler's reagent are added.) Simultaneously with the development of the color in this solution, 1 mgm. of nitrogen (as ammonium sulphate) is Nesslerized in a 100 cc. flask with 25 cc. of the diluted reagent. The flask is made up to the mark, mixed, and a portion poured into one of the cups of the colorimeter and the prism set at 20 mm. The comparison is now made. Since the equivalent of 1 cc. of blood has been employed the calculation is very simple.

3. *Urea.*—In the determination of urea, 10 cc. of the alcoholic filtrate are again employed. This is carefully evaporated to dryness in a similar test tube after the addition of a drop of dilute acetic acid and two or three of kerosene. Folin and Denis add to the residue 2 cc. of 25 per cent. acetic acid, a pebble and 7 grams of dry potassium acetate, and the decomposition is carried out by heating to 153–158°C. for 8 to 10 min., using a calcium chloride tube without bulb as condenser. Benedict's method as described for urine[1] can perhaps be employed more simply. The ammonia is finally removed after the addition of 2 cc. of saturated sodium hydroxide in a similar manner to that described for the total non-protein nitrogen. In this case 10 cc. volumetric flasks are necessary in connection with the Nesslerization for which 3 cc. of the dilute reagent are required. As only 10 cc. of solution are available, a dry colorimeter cup must be at hand. The same standard and similar calculations to the above are employed.

4. *Uric Acid (Folin-Denis)*[2].—The method is based upon the color reaction previously employed by Folin and Macallum[3] for the estimation of uric acid in urine. For the determination, 15 to 25 cc. of blood are employed. The blood is drawn into a small, weighed, wide-mouthed bottle or test tube containing about 0.1 gram of powdered potassium oxalate. The weight of the blood is obtained by difference, and five times the weight of N/100 acetic acid is heated to boiling in an ordinary liter

1. Chapter III, p. 45.

2. Folin and Denis: *Jour. Biol. Chem.*, 1913, XIII, p. 469, also XIV, p. 95.

3. Folin and Macallum: Ibid., 1912, XIII, p. 363.

flask, the oxalated blood added, and the mixture again heated to boiling. The mixture is filtered while still hot. The coagulated blood is stirred up once in about 200 cc. of boiling water, allowed to stand about 5 minutes and filtered through the same filter. The combined filtrate and wash water containing the uric acid and other soluble materials is further acidified with 5 cc. of 50 per cent. acetic acid and evaporated in a suitable porcelain dish to about 3 cc. The liquid is then poured into a 15 cc. centrifuge tube and the dish washed with two successive portions of 0.1 per cent. lithium carbonate solution, using about 2 cc. for each rinsing, any solid material being removed with the aid of a rubber-tipped stirring rod. To the liquid in the centrifuge tube, which should not be greater than 10 cc. in volume, are added 5 drops of 3 per cent. silver lactate solution, 2 drops of magnesia mixture and sufficient strong ammonium hydroxide (10 to 15 drops) to dissolve the silver chloride. The tube is centrifuged for two or three minutes, the supernatant liquid poured off and to the residue are added 4 to 5 drops of fresh saturated hydrogen sulphide water and one drop of concentrated hydrochloric acid. The tube is now placed for a period of five to ten minutes in a beaker of boiling water in order to remove the excess of hydrogen sulphide. Since hydrogen sulphide produces a blue color reaction with the phosphotungstic reagent, it is necessary to remove every trace of this substance. To secure this, a drop of 0.5 per cent. lead acetate is added to the contents of the centrifuge tube as it is taken out of the hot water. (If any blackening should occur, the tube should be heated for another five minutes and another drop of lead acetate added.) The tube is now centrifuged, the supernatant liquid transferred by decantation as completely as possible to a small beaker and the inside of the tube washed with a fine stream of water, care being taken to disturb as little as possible the solid residue in the bottom of the tube. The wash water (should not exceed 5 cc.) is added to the liquid in the beaker and to this acid solution containing the uric acid is then added 2 cc. of the uric acid reagent[1] and 10, 15, or 20 cc. of saturated sodium carbonate solution, depending on whether

1. The reagent is prepared by boiling 100 grams of sodium tungstate and 80 cc. of 85 per cent. phosphoric acid in 750 cc. of distilled water for two hours, preferably under a reflux condenser, and then making up to 1000 cc. with water.

the color obtained requires a final dilution to 25, 50, or 100 cc. Flasks of these capacities should be at hand, and the blue unknown solution is transferred to one of them and diluted with water to the mark. Five cc. of the standard uric acid-formaldehyde reagent[1] are placed in a 100 cc. flask, 2 cc. of the uric acid reagent, 20 cc. of sodium carbonate added, and the solution made up to 100 cc., this being done just previously to the addition of sodium carbonate to the unknown. The latter sometimes needs to be filtered before being transferred to the colorimeter cylinders for the final color comparison. The mm. position at which the standard should be set depends upon individual conditions. Knowing the strength of the standard reagent and the amount of blood employed, the calculation of the uric acid per 100 cc. of blood is not difficult.

The laboratory procedures included in the ordinary routine physical and chemical examination of cerebrospinal fluid,[2] transudates, exudates, etc. include volume, color, specific gravity, reaction, sugar, and protein content, for which the methods described in previous chapters will serve. Occasionally some special examination is required. It may be desired to learn whether or not a fluid is contaminated with urine, and for this purpose the creatinine reaction is appropriate, as it is only in urine that a pronounced test is obtained.

1. This standard is prepared by dissolving one gram of uric acid in 200 cc. of 0.4 per cent. lithium carbonate in a liter flask. Solution is quickly brought about by shaking and 40 cc. of 40 per cent. formaldehyde solution are added and the mixture allowed to stand for a few minutes. The clear solution is acidified by the addition of 20 cc. of normal acetic acid and diluted to the liter mark with water. On the next day, it is standardized against a freshly prepared lithium carbonate solution of uric acid. The color produced by 5 cc. of this solution corresponds very nearly with that obtained with 1 mg. of uric acid. This standard appears to keep permanently.

2. For a consideration of the use of lumbar puncture in children see Pisek: POST-GRADUATE, 1912, XXVII, p. 892.

APPENDIX.

Laboratory Suggestions.

The laboratory suggestions to follow are intended primarily for individuals comparatively unfamiliar with the technique of the various laboratory manipulations. The simple forms of apparatus which have been found of service in the tests previously described are in part shown in the accompanying illustration. With a few exceptions they are inexpensive.

Apparatus.—The uses of the various pieces of apparatus marked in Fig. 13 may be briefly given as follows:

A, screw-top jar, suitable for the collection of gastric contents and feces.

B, urinary sedimenting glass with funnel, convenient for the filtration of gastric contents.

C, porcelain evaporating dish, with 50 cc. burette and stand, used for the titration of gastric acidities, and chlorides and phosphates of urine. The burette is also employed for the titration of urinary acidity and ammonia in connection with the Erlenmeyer flask *K*. The burette, as here illustrated, has a rubber pinchcock. This is to be preferred to a glass stopcock for N/10 NaOH in clinical work, as glass stopcocks are very likely to stick after having been used with alkali, unless very carefully cleaned.

D, small test tube rack with small test tubes, suitable for the estimation of pepsin (Rose's Method), amylase in duodenal juice, etc.

E, small mortar, employed in preparing feces for microchemical examination.

F, fermentation tube for feces, showing vessel *a*, and tubes *b* and *c* as described in Chapter II.

G, test-tube rack designed for urine work. The rack as here illustrated has places for three rows of test tubes, the lower for large tubes (1 inch in diameter) for observation of color, odor, reaction and sp. gr., and from which samples of urine may be withdrawn for the various qualitative tests. The middle row holds the tubes used for the sugar test, and the upper row, a set

of small tubes for Heller's cold nitric acid test for albumin. With this rack, a series of urines may be examined very rapidly without confusion. Where a large number of urines are to be examined, as in hospital work, a series of racks for 12, instead of 6 tubes may be employed.[1]

H, apparatus employed for Benedict sugar titration, consists, of Bunsen burner, tripod with wire gauze, and 25 cc. burette with stand. The 25 cc. burette with glass stopcock is convenient, although the 50 cc. burette might be employed. Where one burette is to be used for all determinations, the 25 cc. instrument as here illustrated is preferable. It should then always be carefully washed out after use, care being taken to see that the stopcock is covered with a thin coat of vaseline.

I, improved Lohnstein saccharimeter.

J, Esbach's albuminometer.

K, Erlenmeyer flask, suitable for titrating urinary acidity and ammonia.

L, liter and two-liter cylinders for making up solutions and measuring volume of urine.

M, Duboscq colorimeter employed in estimating phenolsulphonephthalein, creatinine, indican, and many other substances. For phenolsulphonephthalein, the Hellige instrument, which costs one-fourth as much as the Duboscq, gives satisfactory results.

N, Leitz microscope, without oil immersion lens, but with condenser and iris diaphragm. Very suitable for work in tests previously described, and comparatively inexpensive.

O, cylinder with filter paper in bottom containing a series of pipettes, 1 cc. graduated in 1/100, 1 cc., 5 cc., 10 cc., 25 cc., and 50 cc., and covering all the different quantitative methods.

P, set of dropping bottles, containing various indicators, phenolphthalein, dimethylaminoazobenzene and alizarin.

Q, set of reagent bottles, permanently labeled, very convenient, though not essential.

When it is necessary to make a large number of qualitative tests for sugar in urine, as is generally the case in routine hospital work, the apparatus illustrated in Fig. 14,[2] and employed in connection with Benedict's qualitative solution will be found

1. Cf. Myers: *New York Med. Jour.*, 1913, XCVII, p. 1126.
2. Myers: *loc. cit.*

FIG. 13

FIG. 14

to greatly reduce the time necessary to make the tests without sacrifice to their accuracy. A series of tubes containing Benedict's solution, to which the eight drops of urine have been added and the tubes agitated, are placed in their numerical order in the numbered places of a special copper rack. The rack is then immersed below the upper level of the Benedict solution for two minutes in a bath of saturated calcium chloride, which has just been brought to the boiling point and the flame removed. At the end of the two minutes the rack is elevated, the tubes allowed to drain and cool, and any tubes which show a positive reaction noted.

Form of Reports and Tests Usually Included.—Below are tabulated the forms of report blanks usually employed in the examination of various specimens for diagnostic purposes. In the first column are found the tests which comprise the routine examination, unless otherwise noted. This scheme is convenient for a card index file.

EXAMINATION OF GASTRIC CONTENTS

Name of Patient.....Date.....Age.....Sex.....

Test Meal.....	**Microscopical Examination**
Time Taken.....	Erythrocytes.....
Time Expressed.....	Leucocytes.....
Color.....	Bacteria.....
Consistency.....	Yeasts.....
Odor.....	**Special Examination**
Mucus.....	Blood (chemical).....
Sediment { Quantity.....	Pepsin.....
Sediment { Character.....	Rennin.....
Volume { Total.....	Peptone.....
Volume { Filtrate.....	Starch Products.....
Chemical Examination	Tryptophan Test.....
	Salomon's Test.....
HCl { Free.....	**Remarks**
HCl { Combined.....	.....
Total Acidity.....	.....
Lactic Acid.....	.....

The usual routine in the examination of gastric contents (after the Ewald Meal), as noted above, includes the determination of the acidities, especially free HCl and the total acidity, also the test for lactic acid in the absence of free HCl. The microscopical examination, the test for "occult" blood, the

estimation of pepsin, the tryptophan test and the Salomon test come into consideration in special conditions, such as, *e.g.*, carcinoma of the stomach.

EXAMINATION OF FECES

Name of Patient.....Date.....Age.....Sex.....

Macroscopic Examination	**Special Chemical Examination**
Color.....	Blood.....
Odor.....	Fermentation Test.....
Consistency.....	Schmidt's Reaction.....
Reaction.....	Albumin.....
Mucus.....	Sugar.....
Microscopical Examination	
Connective Tissue.....	Fat.....
Mucus.....	**Remarks**
Muscle Fiber.....	.....
Fat { Globules.....	.....
Fat { Crystals.....	.....
Starch.....	.....
Erythrocytes.....	
Leucocytes.....	
Parasites.....	

The macroscopic examination of feces when properly made is of very great clinical value, often enabling the experienced observer to form a diagnosis. The microscopical examination is of especial value after the intestinal test diet, as indicated in Chapter II. Of the special chemical tests, that for " occult " blood is the most important clinically, though here it is important that the diet from which the feces are obtained should be free from meat.

EXAMINATION OF URINE

Name of Patient.....Date.....Age.....Sex.....

Chemical Examination	**Special Chemical Examination**
Volume in 24 hrs.....	Bile.....
Specific Gravity.....	Blood.....
Color.....	Indican.....
Odor.....	Chlorides as NaCl.....
Reaction.....	Total Nitrogen.....
Sediment.....	Ammonia.....
Albumin.....	Urea.....
Sugar.....	Creatinine.....
Acetone.....	Uric Acid.....
Diacetic Acid.....	Phenolsulphonephthalein.....

Microscopical Examination

.....

.....

Remarks

.....

.....

The usual clinical examination of urine includes the list under *chemical examination* and *microscopical examination* above, except the tests for acetone and diacetic acid.

The tests for acetone and diacetic acid should always be included when sugar has been found to be present. Sugar when detected should be estimated, not simply the percentage elimination determined, but the grams of glucose excreted in twenty-four hours. If an acidosis is present (diabetes, pernicious vomiting of pregnancy, eclampsia, etc.), the degree of this can very easily be gauged by the ammonia elimination.

The quantitative estimation of albumin is often of value in cases of albuminuria, although the quantitative data are not as important here as in glucosuria.

Under the heading of special chemical examinations, in addition to ammonia, those clinically the most important are indican, chlorides, total nitrogen and phenolsulphonephthalein. The indican is often an index as to the amount of a certain type of intestinal putrefaction. The chloride estimation is of value in pneumonia and certain cases of edema. Where it is desired to ascertain the ability of the body to eliminate organic material, the total nitrogen elimination is much to be preferred to the old urea estimation, though it is obvious that a knowledge of the nitrogen content of the diet is a prerequisite. In the cases where it is desired to test the functional capacity of the kidney, the phenolsulphonephthalein test may be of great value.

Recording the examination of milk or other fluids is required of the physician so infrequently that no special mention of them will be made here.

Uniform Charting of Qualitative Tests.—For the sake of uniformity, it is very important to employ a systematic scheme in charting all qualitative reactions, viz., lactic acid in gastric contents, " occult " blood in gastrics and feces, albumin, sugar, acetone, diacetic acid and indican in urine, etc. The following has been found convenient, *faint trace*, *trace*, *small amount*,

moderate amount, *large amount*, and *very large amount*. For microscopical work, especially in the examination of urinary sediments, the following scheme, which will apply to both organized and crystalline sediments, may be employed—*an occasional* (hyaline cast), *a few* (uric acid crystals), *a moderate number* (of pus cells), *many* (finely granular casts), *very many* (calcium oxalate crystals).

CONVENIENT REFERENCE BOOKS.

When more detailed information is desired on the various topics presented in the preceding chapters, the experience of the authors has shown the following books to be particularly useful.

For the general application of chemical findings to diagnosis:

Simon: Clinical Diagnosis.
Wood: Chemical and Microscopical Diagnosis.

For the presentation of various phases of digestion and nutrition:

Howell: Text-book of Physiology.
Hammarsten-Mandel; Text-book of Physiological Chemistry.
Lusk: Science of Nutrition.
Sherman: The Chemistry of Food and Nutrition.
Taylor: Digestion and Metabolism.
Krehl-Hewlett: Clinical Pathology.
von Noorden-Hall: Metabolism and Practical Medicine.

For a discussion of the diseases of the stomach and intestines, and of the composition of the feces:

Cohnheim (P.)—Fulton: Diseases of the Digestive Canal.
Einhorn: Diseases of the Stomach.
Einhorn: Diseases of the Intestines.
Schmidt-Aaron: Test Diet in Intestinal Diseases.
Schmidt und Strasburger: Die Fäzes des Menchen.

For further details on modern quantitative methods of urine analysis:

Hawk: Practical Physiological Chemistry.

For a discussion of diabetes:

Cammidge: Glycosuria and Allied Conditions.
von Noorden: New Aspects of Diabetes.

For a consideration of milk and its relation to infant nutrition:

Langstein und Meyer: Säuglingsernährung und Säuglingsstoffwechsel.

INDEX.